Original article
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Phylogenetic status of a lung fluke in the Philippines based on mitochondrial genome

YUKITA SATO¹, MORITOSHI IWAGAMI², JUN IWASHITA², TATSUYA ABE², MASAYOSHI YUKAWA¹,
BAYANI L. BLAS³, KENJIRO KAWASHIMA² AND TAKESHI AGATSUMA²*

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Abstract

Based on a near-complete mitochondrial DNA sequence, the phylogenetic status of a lung fluke collected in the Philippines was evaluated. The lung fluke from Leyte Island, Philippines, resembles Paragonimus westermani morphologically and is sometimes regarded as a subspecies, P. westermani filipinus. In this study, all mitochondrial genes of the Leyte form were sequenced: 12 subunits of mitochondrial enzymes, 2 ribosomal RNA genes and 21 transfer RNAs. The gene order is the same as that of the previously-published P. westermani from Korea. All genes are transcribed in the same direction. The sequence from Leyte was 88.8% identical to the previously-published sequence (accession No. AF219379). This is further evidence that the Philippine form should be regarded as specifically distinct from the form in East Asia (China, Japan and Korea). This conclusion is strengthened by the observation that the molluscan host of the Philippine form is of a different family from that of the East Asian form.

Key words: mt DNA, complete sequence, Paragonimus westermani, P. filipinus, Philippines

INTRODUCTION

The lung fluke, Paragonimus westermani (Kerbert, 1878) is a well known zoonotic agent found in eastern and southern Asia and has been the focus of many taxonomic and biological studies (e.g. Blair et al., 1999). Several previous studies have suggested that some regional populations of P. westermani should be regarded as distinct species. For example, Miyazaki (1981) proposed on morphological grounds that the Philippine form should be recognized as a distinct subspecies, P. westermani filipinus. Allozyme analysis and partial mitochondrial DNA sequences demonstrated that Philippine isolates are genetically distant from strains found in Taiwan, Japan and North East China (Agatsuma et al., 1988; Blair et al., 1997). Previous analyses of partial sequences (e.g. Iwagami et al., 2000) used only short sequence tracts. Comparisons of complete genome sequences can provide more reliable results and also permit comparisons of features such as gene order and the structure of non-coding regions. In this study, we present and discuss the complete sequence of the coding region of the mitochondrial genome for the Philippine lung fluke. In the light of this, we make recommendations concerning its phylogenetic status.

MATERIALS AND METHODS

Adult specimens of a Philippine species of Paragonimus used in this study are those previously reported (Agatsuma et al., 1988). DNA extraction of the specimens is as described previously (Iwagami et al., 2000). Based on the 14,967bp of mitochondrial DNA sequence from P. westermani (Korean triploid form - database accession No. AF 219379), several PCR primer sets were designed and applied for the present study. Amplified PCR products were purified by gel-electrophoresis and ethanol-precipitation, ligated into pGEM-T plasmid vector (Promega) and cloned inserts sequenced using a dye terminator cycle-sequencing kit (Applied Biosystems) and an automated sequencer (ABI 310, Applied Biosystems). Multiple sequence alignments were performed using the programs CLUSTAL V and GENETYXMAC.

RESULTS AND DISCUSSION

In total, 14,199bp were amplified and sequenced. As
shown in Fig. 1, the putative mitochondrial genes included 12 subunits of mitochondrial enzymes (ATP6, CO1\(\sim\)3, Cytb, ND1\(\sim\)6, ND4L), two ribosomal RNA genes (12S rRNA and 16S rRNA) and 21 transfer RNAs (tRNA). As is the case in other trematodes, serine and leucine are each specified by two different tRNAs. No tRNA corresponding to glutamic acid (Glu: E) was found. The gene order and direction of transcription was determined by comparison with the previously reported sequences (Le et al., 2000).

Sequence comparisons between Philippine (2n) and Korean (3n) forms are shown in Table 1. The overall difference in DNA sequences of protein-coding genes was 12.40%, in tRNA genes 6.10% and in ribosomal RNAs, 8.03%.

The complete mitochondrial genome of *P. westermani* is estimated to be about 21Kbp (Agatsuma et al., 1994). Of the 14,199 bp we sequenced, 13,196 bp coded for genes and 1,003 bp consisted of non-coding regions. The portion remaining to be sequenced is therefore about 6,800 bp and must constitute the long variable non-coding region (VNCR). Complete sequencing of the VNCR has not been possible to date, probably because of the presence of many repeats. This region corresponds to origin of replication of mitochondrial genomes in many other organisms, attracting interest as to why the length of the site in *Paragonimus* seems to be much longer than in others.

### Table 1. Sequence analysis of the mitochondrial genome of a lung fluke from the Philippines, compared to a triploid type of *Paragonimus westermani* from Korea

<table>
<thead>
<tr>
<th>Protein-coding region</th>
<th>Number of bases</th>
<th>Number of substitution</th>
<th>Rate of difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP6</td>
<td>513</td>
<td>62</td>
<td>12.09</td>
</tr>
<tr>
<td>CO1</td>
<td>1,536</td>
<td>151</td>
<td>9.83</td>
</tr>
<tr>
<td>CO2</td>
<td>600</td>
<td>62</td>
<td>10.33</td>
</tr>
<tr>
<td>CO3</td>
<td>645</td>
<td>70</td>
<td>10.85</td>
</tr>
<tr>
<td>Cytb</td>
<td>1,119</td>
<td>126</td>
<td>11.26</td>
</tr>
<tr>
<td>ND1</td>
<td>891</td>
<td>114</td>
<td>12.79</td>
</tr>
<tr>
<td>ND2</td>
<td>867</td>
<td>131</td>
<td>15.11</td>
</tr>
<tr>
<td>ND3</td>
<td>357</td>
<td>47</td>
<td>13.17</td>
</tr>
<tr>
<td>ND4</td>
<td>1,263</td>
<td>180</td>
<td>14.25</td>
</tr>
<tr>
<td>ND4L</td>
<td>258</td>
<td>18</td>
<td>6.98</td>
</tr>
<tr>
<td>ND5</td>
<td>1,584</td>
<td>225</td>
<td>14.20</td>
</tr>
<tr>
<td>ND6</td>
<td>453</td>
<td>65</td>
<td>14.35</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10,086</strong></td>
<td><strong>1,251</strong></td>
<td><strong>12.40</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>tRNA</th>
<th>Number of bases</th>
<th>Number of substitution</th>
<th>Rate of difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>73</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arg</td>
<td>72</td>
<td>5</td>
<td>4.17</td>
</tr>
<tr>
<td>Asn</td>
<td>71</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Asp</td>
<td>67</td>
<td>6</td>
<td>7.46</td>
</tr>
<tr>
<td>Cys</td>
<td>64</td>
<td>6</td>
<td>9.23</td>
</tr>
<tr>
<td>Gin</td>
<td>62</td>
<td>1</td>
<td>1.61</td>
</tr>
<tr>
<td>Gly</td>
<td>71</td>
<td>4</td>
<td>5.71</td>
</tr>
<tr>
<td>His</td>
<td>62</td>
<td>11</td>
<td>12.70</td>
</tr>
<tr>
<td>Ile</td>
<td>62</td>
<td>1</td>
<td>1.61</td>
</tr>
<tr>
<td>Leu (CUN)</td>
<td>62</td>
<td>0</td>
<td>1.61</td>
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<tr>
<td>Leu (UUN)</td>
<td>65</td>
<td>3</td>
<td>4.62</td>
</tr>
<tr>
<td>Lys</td>
<td>65</td>
<td>3</td>
<td>7.58</td>
</tr>
<tr>
<td>Met</td>
<td>66</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phe</td>
<td>70</td>
<td>6</td>
<td>8.57</td>
</tr>
<tr>
<td>Pro</td>
<td>66</td>
<td>10</td>
<td>13.43</td>
</tr>
<tr>
<td>Ser (AGN)</td>
<td>60</td>
<td>5</td>
<td>8.33</td>
</tr>
<tr>
<td>Ser (UCN)</td>
<td>65</td>
<td>2</td>
<td>3.08</td>
</tr>
<tr>
<td>Thr</td>
<td>66</td>
<td>3</td>
<td>4.55</td>
</tr>
<tr>
<td>Trp</td>
<td>64</td>
<td>4</td>
<td>6.25</td>
</tr>
<tr>
<td>Tyr</td>
<td>62</td>
<td>3</td>
<td>4.84</td>
</tr>
<tr>
<td>Val</td>
<td>63</td>
<td>10</td>
<td>15.38</td>
</tr>
<tr>
<td>Glu</td>
<td>Not found</td>
<td>Not found</td>
<td>Not found</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,378</strong></td>
<td><strong>84</strong></td>
<td><strong>6.10</strong></td>
</tr>
</tbody>
</table>

*Accession No. AF219379
As mentioned above, Miyazaki (1981) regarded the Philippine lung fluke as a subspecies of Paragonimus westermani, namely P. westermani filipinus. The justification was that one lobe of one or both testes is detached from the remainder and that the molluscan host belongs to a different family from that of P. westermani. Blair (2000) reviewed phylogenetic relationships among species and strains of the genus Paragonimus and showed P. westermani filipinus to be distinct from populations from Japan, Taiwan and Malaysia. His study used only 393 bp of the mitochondrial CO1 gene. Partial CO1 sequences previously deposited in the public databases showed 88.7% identity between Paragonimus isolates from the Philippines and Korea, suggesting that Paragonimus in the Philippines can be regarded as a species distinct from P. westermani from East Asia (China, Japan and Korea). However this suggestion was thought to be weak because such short sequence tracts were used. Our results enabled full comparison of all genes of the mitochondrial genome for the first time and found an overall identity of 88.8% between Philippines and Korean worms (Table 1). This result resembles that of the partial sequence analysis discussed above. Despite the lack of clear molecular criteria to determine species-boundaries within a genus such as Paragonimus, we consider that our results, and the differences in molluscan host specificity, are good evidence that the Philippine form is specifically distinct. The name P. filipinus Miyazaki, 1981 should be applied to it.

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Phylogenetic relationships of snails of the genera *Oncomelania* and *Tricula* inferred from the mitochondrial 12S rRNA gene

MUNEHIRO OKAMOTO, CHIN-TSON L, WILFRED U. TIU, DONGCHUAN QUI, PINARDI HADIDJAJA, SUCHART UPATHAM, HIROMU SUGIYAMA, TAKAHIRO TAGUCHI, HIROHISA HIRAID, YASUHIDE SAITO, SHIGEHISA HABE, MASANORI KAWANAKA, MIZUKI HIRATA AND TAKESHI AGATSUMA

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Abstract

The *Schistosoma japonicum* group and *S. sinensium* utilize intermediate snail hosts belonging to the genera *Oncomelania* and *Tricula* (Gastropoda: Pomatiopsidae). In the present study, partial sequences of the mitochondrial 12S rRNA gene from 7 subspecies of *O. hupensis*, two species of *Tricula* (*T. bollingi* and *T. humida*) and *O. minima* were examined to infer a phylogeny for these. Nucleotide differences among subspecies of *O. hupensis* were less than 6.5% and among species from different genera, 10-12%. The phylogenetic tree obtained in this study indicates that *O. hupensis* subspecies fell into four distinct clades; that is, *O. h. quadrasi* from the Philippines, *O. h. lindoensis* from Indonesia, *O. h. hupensis* from Yunnan, China and the remaining 5 subspecies (*O. h. robertsoni* from China, *O. h. formosana* from Taiwan, *O. h. chiui* from Taiwan and *O. h. nosophora* from Japan). The phylogenetic tree also showed that *O. minima* was placed as sister to all of the subspecies of *O. hupensis*. Possible evolutionary relationships among the snail hosts were discussed.

Key Words: *Oncomelania, Tricula*, mitochondrial DNA, 12S rRNA gene, phylogenetic tree

INTRODUCTION

Species of *Schistosoma* have been placed in a number of groups based on, amongst other things, egg morphology and/or geographical distribution (Rollinson and Southgate, 1987). For example, African schistosomes of the *S. mansoni* and *S. haematobium* groups develop in pulmonate snails of the family Planorbidae, while the oriental species, namely the *S. japonicum* group (*S. japonicum, S. mekongi, S. malayensis*) and *S. sinensis*, utilize snails belonging to the family Pomatiopsidae. Pulmonate snails belong to the subclass Pulmonata, while pomatiopsids belong to the subclass Caenogastropoda. These two subclasses diverged a long time ago (Davis, 1980). Generally, specificity for an intermediate host in the genus *Schistosoma* is high (Rollinson and Southgate, 1987). So the phylogeny of these intermediate host snails seems to be very important when the evolution of the schistosome is traced.

*Schistosoma japonicum* is widely distributed throughout East Asia, China and Japan, and the intermediate host is *Oncomelania hupensis*. Since *O. hupensis* shows geographical variation in morphology, many subspecies have
been described (Davis et al., 1995). Geographical variation is so great that some researchers prefer to regard the subspecies as independent species (Woodruff et al., 1988; Nihei et al., 1998). In the present study, phylogenetic relationships of the snail hosts of S. japonicum, O. hupensis, and other related species of Oncomelania and Tricula were studied using the mitochondrial 12S rRNA gene.

**MATERIALS AND METHODS**

**Snail samples**

Sixteen isolates of O. hupensis, belonging to 7 subspecies, were examined. We also examined O. minima, T. bol-lingi and T. humida. The geographical origins and locations of species or subspecies used in this study are shown in Table 1 and Fig. 1.

**Preparation of DNA**

Genomic DNA from each snail sample was extracted using Easy-DNA Kit (Invitrogen, USA). DNA extracted by this kit contained an inhibitor for PCR, and this inhibitor could not be removed by phenol/chloroform extraction or commercially available spin column etc. So DNA was purified by 0.5% agarose gel electrophoresis with 0.5 X Tris-boric EDTA buffer. After electrophoresis, high molecular weight DNA was cut off with agarose gel and extracted by QIAEX II Gel Extraction Kit (Qiagen, Germany).

**Amplification and sequencing of DNA**

Purified genomic DNA was used as a template for amplification of DNA fragments by the polymerase chain reaction (PCR). Amplification of a part of the mitochondrial 12S RNA gene was carried out using universal primers (Kocher et al., 1989). The sequences of the primers were as follows: L1091; 5'-AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT ATT-3' and H1478; 5'-TGA CTG CAG AGG GTG ACG GGC GGT GTG T-3'. PCR was performed using Ampli Taq DNA Polymerase (Perkin Elmer, USA) according to the manufacturer’s instructions. After an initial denaturation step (94°C for 3 minutes) there were 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 1 min. PCR products were purified with QIAquick-spin PCR purification Kit (Qiagen). Purified double-stranded PCR products were directly sequenced with the same primers as those of PCR from both ends using Dye Terminator Cycle Sequencing FS Ready Reaction Kit and a Model 377A DNA sequencer (Perkin Elmer).

**Phylogenetic analyses**

DNA sequence data were aligned using the CLUSTAL W computer program. The evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980), and the phylogenetic tree was constructed by the neighbor-joining method using the neighbor-joining computer program in the PHYLIP 3.5 phylogeny package (Felsenstein, 1993). The tree was evaluated using the bootstrap test based on 1,000 resampling. A sequence from Littorina littorea was used for the outgroup (Rumbak et al., 1994).

**RESULTS AND DISCUSSION**

Using this primer pair, fragments of 365 to 368 bp were amplified in this study. Sequences and partial alignments of 12S rRNA gene are shown in Fig. 2. The maxi-
Fig. 2. Nucleotide sequence alignment of the 12S ribosomal RNA gene in the mitochondrial DNA in the three genera, *Oncomelania*, *Tricula* and *Littorina*.

### Table 2. Pairwise differences in nucleotide sequences of the 12S rRNA gene among subspecies/species of the genus *Oncomelania* and *Tricula*.

<table>
<thead>
<tr>
<th>species/subspecies</th>
<th>O.h.hupen.</th>
<th>O.h.h.Yun</th>
<th>O.h.rob.</th>
<th>O.h.chi</th>
<th>O.h.nos.</th>
<th>O.h.lin.</th>
<th>O.h.qua.</th>
<th>O.minima</th>
<th>T.bollingi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. hupensis</em> (excluding Yunnan)**</td>
<td>0.8*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. hupensis</em> (only Yunnan)**</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. robertsoni</em></td>
<td>0.7</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. formosana</em></td>
<td>1.1</td>
<td>3.7</td>
<td>1.0</td>
<td>1.3*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. chiui</em></td>
<td>0.0</td>
<td>4.6</td>
<td>0.8</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. nosophora</em></td>
<td>1.7</td>
<td>4.8</td>
<td>1.6</td>
<td>2.0</td>
<td>1.8</td>
<td>0.7*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. lindoensis</em></td>
<td>3.6</td>
<td>6.4</td>
<td>3.4</td>
<td>3.4</td>
<td>4.2</td>
<td>0.8*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. quadriasi</em></td>
<td>5.1</td>
<td>6.4</td>
<td>5.3</td>
<td>5.0</td>
<td>5.0</td>
<td>6.1</td>
<td>6.2</td>
<td>0.7*</td>
<td></td>
</tr>
<tr>
<td><em>O. minima</em></td>
<td>9.4</td>
<td>9.3</td>
<td>9.6</td>
<td>9.3</td>
<td>9.3</td>
<td>9.1</td>
<td>8.3</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>T.bollingi</td>
<td>10.0</td>
<td>10.2</td>
<td>10.2</td>
<td>10.4</td>
<td>10.4</td>
<td>9.9</td>
<td>9.8</td>
<td>11.7</td>
<td>8.8</td>
</tr>
<tr>
<td>T.humida</td>
<td>10.7</td>
<td>12.1</td>
<td>11.0</td>
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<td>10.7</td>
<td>10.5</td>
<td>10.8</td>
<td>12.5</td>
<td>9.0</td>
</tr>
</tbody>
</table>

*: values for intra-subspecies

**: The Yunnan strain of *O. hupen.* was listed separately from the other strains, because it differed in a high degree from any other subspecies of *O. hupensis.*
imum levels of nucleotide variations detected between pairs of species or subspecies for the 12S rRNA gene are shown in Table 2. The values are expressed as pairwise differences in percentage. Nucleotide differences within the subspecies of *O. hupensis* were in general very low. However, in the case of *O. h. hupensis*, the intra-nucleotide difference was very large, because a Yunnan isolate of *O. h. hupensis* differed from all of the other isolates. On the other hand, differences among five of the subspecies, *O. h. hupensis* from China except for Yunnan, *O. h. robertsoni*, *O. h. formosana*, *O. h. chui* and *O. h. nosophora*, were less than 2.0%. Nucleotide differences between these 5 subspecies and *O. h. lindoensis*, or *O. h. quadrasi* or the Yunnan isolate of *O. h. hupensis* were larger, being about 3 to 6%. Very large values of nucleotide differences (about 9 to 12%) were obtained between different species.

As shown in Fig. 3, a phylogenetic tree of the snail hosts was constructed using the neighbor-joining method. *Oncomelania hupensis* subspecies are distributed among 4 groups. That is, four specimens of *O. h. quadrasi* from Philippines form a monophyletic clade. *Oncomelania h. nosophora*, *O. h. hupensis*, *O. h. robertsoni*, *O. h. chui* and *O. h. formosana* form one group. *Oncomelania h. lindoensis* and *O. h. hupensis* from Yunnan make independent clades. In the meantime, *O. minima*, *T. bollingi* and *T. humida* also make independent clades, and they are genetically distant from each other as well as from all the subspecies of *O. h. hupensis*.

The sequence of mitochondrial 12S rRNA gene has been utilized to infer the phylogeny of various animals (Ko-cher et al., 1989). Rumbak et al. (1994) examined the phylogenetic relationships among 11 species in the genus *Littorina*, which is a widely distributed marine gastropod, belonging to the same suborder Archaeoetaenioglossa as the genus *Oncomelania*. In the case of the genus *Littorina*, nucleotide differences between European species and American species were about 3%. And differences between subgenera in the genus *Littorina* were about 5% or more. In the present study, *O. hupensis* were distributed among 4 groups, and nucleotide differences among these 4 groups were 3 to 6%. Although there is no reason to assume that rates of molecular evolution have been the same in *Littorina* as in the pomatiopsids, comparisons of the percentage differences between the two studies suggest that the subspecies in *O. hupensis* may require re-evaluation as suggested by Woodruff et al. (1988). Despite the wide geographic area involved, the genetic differences between the subspecies in Japan, Taiwan and China were very small. All of the examined snails in China were collected from the Yangtze basin except for that of Yunnan Province. In the glacial maxima, only approximately twenty thousand years ago (Wang and Sun, 1994), sea levels were much lower than now, the mouth of the Yangtze was considerably closer to Japan and Taiwan, and the Taiwan channel was dry land. This could explain the high levels of similarity among Japanese, Taiwanese and Yangtze basin samples.

It has been reported that genetic variation among *S. japonicum* populations in Asia, including China, the Philippines, Japan and Indonesia, is very slight (Bowles et al., 1993). However, we have shown that genetic variation among their intermediate host snails is quite considerable. Thus, our study supports the idea that *S. japonicum* has been recently introduced to many areas where it now occurs, and has been able to adapt to local strains of *Oncomelania*, as suggested in previous papers (Woodruff, 1988; Davis, 1992; Atwood et al., 2002).

Davis (1980) suggested that *S. sinensis* may constitute a species complex, because of geographical differences in snail host specificity. Our previous studies showed a large difference in egg sizes as well as in nucleotide sequence of CO1 between the two isolates of *S. sinensis* from China and Thailand, supporting Davis’ hypothesis (Kawanaka et al., 1998; Agatsuma et al., 2000). The present result of a large distance value between their intermediate hosts may suggest coevolution between the hosts and parasites.

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PROCEEDINGS OF THE 43RD ANNUAL MEETING OF
JAPANESE SOCIETY OF TROPICAL MEDICINE

21-22 November 2002, Kochi

President
Yoshihisa Hashiguchi
Professor, Department of Parasitology
Kochi Medical School

CONTENTS

Prize winner’s lecture
JSTM (Japanese Society of Tropical Medicine) Young Investigator Award
The malaria vector in the area surrounded by rice field in Khammaoune Province, Lao PDR with
the discussion of the future ITM strategy in Laos and also Mekong
Kobayashi, J.

President’s lecture
Tropical diseases research and the endemic areas--- A brief review, based on the research/control of
leishmaniasis in South and Central Americas
Hashiguchi, Y.

Special lectures
I. The mucosal immune system for the development of new generation vaccine
Kiyono, H.
II. Molecular strategies for disease control--- research trials in vector biology---
Chinzei, Y.

Educational lecture
The great names of history in tropical medicine
Kobayashi, T.

Symposium
Tropical medicine and emerging/re-emerging infectious diseases
S1I01 Current status of emerging protozoal infectious diseases: cryptosporidiosis, cyclosporiasis, and microsporidiosis
Iseki, M.
S1I02 Cryptosporidiosis and cyclosporiasis: the clinical features and occurrence in Japan
Masuda, G.
S1I03 Emerging and re-emerging arboviral infectious diseases
Takasaki, T. and Kurane, I.
S1I04 Current status of emerging and re-emerging bacterial infectious diseases
Nagatake, T.

Leishmaniasis: from bench to field
S2I01 Detection of new endemic areas of cutaneous leishmaniasis in Pakistan: a six-year study
Bhutto, A. M. et al.
S2I02 Current status of leishmaniasis in Ecuador
Calvopina, M. and Hashiguchi, Y.
S2I03 Evolution of the cytochrome b gene of Leishmania and Endotrypanum
Uezato, H. et al.
S2I04 Advance in development of anti-leishmanial drugs and research on mechanisms of drug resistance in Leishmania
Katakura, K. et al.

For the fruits of tropical medicine
S3I00 For the fruits of tropical medicine
Hamano, S.
S3I01 Synthesis of useful antibody molecules using the combinatorial library and the phage display technologies
Yamashiro, T.
S3I02 Molecular mechanism of malarial sporozoites infection into the mammalian liver
Ishino, T. et al.
S3I03 Involving tropical medicine practically
Awazawa, T.
S3I04 What is the tropical medicine in Japan? --- A question from a young scientist
Kaneko, O.
Hansen’s disease
S 01 Adequate treatment and early diagnosis of leprosy Hosokawa, A. et al.
S 02 Around the fundamental leprosy research Sasaki, S.
S 03 Present situation of Hansen’s disease in the world Ishii, N.
S 04 Present situation of leprosy diseases in Myanmar Soe, T. A.
S 05 Activities of JICA project for leprosy control and basic health services in Myanmar Nagao, E.

Tropical medicine in the scope of international contribution
S 01 Role of the JICA Zambia project in AIDS control in Southern Africa Terunuma, H.
S 02 Tuberculosis control in Nepal and Japanese medical cooperation Osuga, K.
S 03 Problems of implementing the malaria control project Kanbara, H.
S 04 Hashimoto initiative and Okinawa infectious disease initiative: Japan’s challenge to global parasite control
S 05 The progress and the direction of the Asian Center of International Parasite Control Project Nagai, N. et al.
S 06 Roles and challenges of Japan’s ODA for global health Kunii, O.

Looking for ideal fieldworks in tropical medicine
S 01 Difficulties encountered in field researches which were used as excuses Shimada, M.
S 02 From Voodoo epidemiology to reliable epidemiology Yoshimura, T.
S 03 Filariasis control: how were blood collection and mass drug treatment done? Kimura, E.
S 04 Bioethics and biomedical research in developing countries: What should researchers do from the viewpoint of bioethics? Kaneko, S.

Workshop
W 01 01 Preventive behavior and morbidity of Japanese tourists for Africa Kikuchi, H. et al.
W 01 02 Knowledge and preparation regarding the risk of malaria among Japanese international travelers Kawakami, K. et al.
W 01 03 Disease trends among Japanese people living in Southeast Asia Uchikoshi, A. et al.
W 01 04 The storage and supply system of orphan drugs for tropical and parasitic diseases Kimura, E. et al.
W 02 01 Molecular analysis of α-thalassemia in Nepal: Correlation with malaria endemicity. ( II ) Hamano, M. et al.
W 02 02 Study on DNA polymorphism in the DHFR and DHPS genes of Plasmodium falciparum in southern Laos Imada, Y. et al.
W 02 03 Molecular analysis of chloroquine resistant related gene in Malawi Mita, T. et al.
W 02 04 Generation of transgenic anopheline mosquitoes expressing a gene encoding anti-malaria molecule Yoshida, S. et al.
W 02 06 Intranasal inoculation of yeast-produced malaria ookinate surface proteins co-administered with cholera toxin induces complete transmission-blocking immunity against a rodent and a human malaria parasite Kishimoto, A. et al.
W 02 07 High expression of cholera toxin B subunit pentamer in methanophilic yeast Pichia pastoris and its oral immunogenicity in mice Arakawa, T. et al.
W 02 08 Expressions of cytokines and monosaccharide transporters in the duodenal mucosa of gastro-intestinal symptomatic patients in the rural area of Thailand Lertanekawattana, S. et al.
W 03 01 Study of Ascaris and ulcerative colitis----Genetic analysis of cross-reactive antigenicity between Ascaris lumbricoides homeinis antigen and human colon mucosa Hayashi, I. et al.
W 03 02 Fusion DNA vaccine against infection with high virulent Toxoplasma Ishii, K. et al.
W 03 03 An interleukin-18 expression plasmid administration initiates a protective T helper type 1 immune response and resolves cutaneous leishmaniasis Li, Y. et al.
W 03 04 Protective role of IgE in mice infected with Plasmodium berghei ANKA Furuta, T. et al.
W 03 05 The role of erythrocytes on suppression of immune response inmurine malaria Tokunaga, M. et al.
W 03 06 Application of questionnaires for monitoring Schistosoma mansoni infection after mass-treatment Kisu, T. et al.
W 03 07 Rapid school health questionnaires surveys on urinary schistosomiasis in coastal Kenya and Zanzibar Moji, K. et al.
<table>
<thead>
<tr>
<th>Paper ID</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>W023</td>
<td>Water contact behaviors among schoolchildren in an endemic area of schistosomiasis, Tanzania: A comparative study of direct observation and 24-hour recall method</td>
<td>Ataka, Y. et al.</td>
</tr>
<tr>
<td>W024</td>
<td>School-based and residence-based analyses on infections with S. haematobium and S. mansoni among children in Moshi, Tanzania</td>
<td>Kaneda, E. et al.</td>
</tr>
<tr>
<td>W021</td>
<td>Characterization of the cDNA of the miracidial antigen family of Schistosoma japonicum (Chinese mainland strain)</td>
<td>Yu, C. et al.</td>
</tr>
<tr>
<td>W022</td>
<td>Molecular cloning of a novel procyclic form-specific gene (cDNA) from Trypanosoma brucei</td>
<td>Oshima, S. et al.</td>
</tr>
<tr>
<td>W023</td>
<td>Analysis of Leishmania amastigote stage-specific molecules</td>
<td>Sanjoba, C. et al.</td>
</tr>
<tr>
<td>W024</td>
<td>The quantification of the genes involved NO production in Leishmania-infected macrophage by real-time PCR</td>
<td>Yamamoto, Y. et al.</td>
</tr>
<tr>
<td>W022</td>
<td>The induction of TNF-α and iNOS expression by macrophage cells against ONO-4007 (a synthetic lipid A derivative), IFN-γ and meglumine antimoniate in vitro study</td>
<td>Khan, M. A. K., et al.</td>
</tr>
<tr>
<td>W023</td>
<td>Marine sponges: hopeful sources of new anti-protozoan drugs</td>
<td>Goto, Y. et al.</td>
</tr>
<tr>
<td>W024</td>
<td>Combination effects of chloroquine with febrifugine/isofebrifugine mixture against chloroquine-resistant Plasmodium berghei NK65 in ICR mice</td>
<td>Ishii, A. et al.</td>
</tr>
</tbody>
</table>

**General presentation**

<table>
<thead>
<tr>
<th>Paper ID</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>B001</td>
<td>Clinical features of mucocutaneous leishmaniasis in the Amazonian region of Ecuador</td>
<td>Calvopina, M., Hashiguchi, Y.</td>
</tr>
<tr>
<td>B002</td>
<td>Malaria epidemiology in Lombok Island, Indonesia</td>
<td>Kanbara, H. et al.</td>
</tr>
<tr>
<td>B003</td>
<td>Babesia sp. And other hemoparasites identified in wild rats in Chiang Mai and Mae Hong Son Province, Thailand</td>
<td>Dantrakool, A. et al.</td>
</tr>
<tr>
<td>B004</td>
<td>Adult black flies attracted to humans and water buffaloes, and natural infections with Onchocerca larvae in Chiang Mai, in Northern Thailand</td>
<td>Takaoka, H. et al.</td>
</tr>
<tr>
<td>B005</td>
<td>Seasonal changes of wild anopheline mosquitoes around Jichi Medical School</td>
<td>Matsuoka, H. et al.</td>
</tr>
<tr>
<td>B007</td>
<td>Mitochondrial quinol-fumarate reductase (complex II) from Ascaris suum as a target for chemotherapy</td>
<td>Oshashi, M. et al.</td>
</tr>
<tr>
<td>B008</td>
<td>Chemotactic factors for leukocytes from eggs of Schistosoma mekongi</td>
<td>Ohashi, M. et al.</td>
</tr>
<tr>
<td>B009</td>
<td>Characterization of recombinant fragments of the 150-kDa lectin (IGL) of Entamoeba histolytica</td>
<td>Tachibana, H. et al.</td>
</tr>
<tr>
<td>B010</td>
<td>Involvement of proteasome function in Entamoeba growth and differentiation</td>
<td>Makioka, A. et al.</td>
</tr>
<tr>
<td>B011</td>
<td>Molecular cloning and characterization of farnesyltransferase of Entamoeba histolytica</td>
<td>Makioka, A. et al.</td>
</tr>
<tr>
<td>B013</td>
<td>A mechanism of thrombocytopenia during the acute phase of dengue virus infection: Involvement of platelet-associated IgG and anti-dengue virus IgG</td>
<td>Oishi, K. et al.</td>
</tr>
<tr>
<td>B014</td>
<td>Evaluation of a hepatitis B vaccination program in Chiang Mai, Thailand</td>
<td>Toriyama, K. et al.</td>
</tr>
<tr>
<td>B016</td>
<td>A trial of nosocomial infection control in the Bach Mai Hospital Project for functional enhancement in Vietnam</td>
<td>Ohara, H., Hung, N. V.</td>
</tr>
<tr>
<td>B017</td>
<td>Experimental infection into SCID mice with microsporidia isolated from AIDS patient</td>
<td>Juarez S. I. et al.</td>
</tr>
<tr>
<td>C001</td>
<td>Analysis of antigen-specific IgG subclasses in serum and urine samples from bancroftian filariasis patients</td>
<td>Qi, X. -G. et al.</td>
</tr>
<tr>
<td>C002</td>
<td>Direct agglutination test (DAT) with urine samples for the diagnosis of visceral leishmaniasis</td>
<td>Islam, M. Z. et al.</td>
</tr>
</tbody>
</table>
C004 Anti-SERA antibody production as a parameter of anti-malaria immunity in individuals in the Solomon Islands
Hato, M. et al.

C005 Serodiagnosis of tuberculosis using MPB64 derived from Mycobacterium bovis
Yoshidome, A. et al.

C006 A method of blood examinations in overseas mobile clinic--- its clinical application to the remote areas in Lao PDR
Ishida, T. et al.

C007 Comparison of ultrasonographic images between Schistosoma japonicum and S. mekongi infection
Ohmae, H. et al.

C008 Comparison of ultrasonographic images of liver and spleen by age group and the relationship between frequency of praziquantel treatment and ultrasonographic images of patients suffering from Schistosoma japonicum and S. mekongi infection in the Philippines and in Cambodia
Chigusa, Y. et al.

C009 The influence of Schistosoma haematobium infection on voiding function
Watanabe, K. et al.

C010 Epidemiological surveillance on schistosomiasis in mountainous regions in Sichuan
Chuang, C. -K. et al.

C011 Parasitological survey and related health awareness investigation by questionnaire at a village in the central region in Ghana
Osada, Y. et al.

C012 Damage in cercariae of Schistosoma mansoni induced by pressure jar in water
Ohwatari, N. et al.

C013 Comparison of intestinal parasitic infections and malnutrition in schoolchildren in urban and rural areas in Hanoi, Vietnam
Fujimaki, Y. et al.

C014 Detection of microorganisms in tap water in Indonesia and Thailand
Uga, S. et al.

C015 Epidemiological analysis of enterohemorrhagic Escherichia coli infection in Indonesia
Ichinose, Y. et al.

C016 The problem on malaria treatment--- About the delay of diagnosis and treatment
Okumura, E. et al.

C017 A severe case of Plasmodium falciparum infection complicated with black water fever
Koyama, K. et al.

C018 Correlation between the recognition by obstetricians about congenital toxoplasmosis and their clinical activities
Naoi, K. et al.
Prize winner’s lecture

THE MALARIA VECTOR IN THE AREA SURROUNDED BY RICE FIELD IN KHAMMAOUNE PROVINCE, LAO PDR WITH THE DISCUSSION OF THE FUTURE ITM STRATEGY IN LAOS AND ALSO MEKONG AREA

JUN KOabayashi

Bureau of International Cooperation, International Medical Center of Japan

In the 1996, the malaria vector in Laos was not clear. It was suspected that the same vector in the North Thailand; Anopheles minimus, An. maculatus and An. dirus, were existed. In Nhommarath District in Khammouane Province, Lao PDR, a lot of malaria patients were reported in the district hospital located in the center of basin. It was supposed that patient came from the communities around hospital under the consideration of accessibility to hospital, thus the one community around hospital was selected for malaria survey. The prevalence of malaria infection in the inhabitant is high as more than 20%, however, only few number of An. minimus was collected, and An. maculates and An. dirus was not collected. On the other hand, An. nivipes is the majority in Anopheles, which was collected by human bait collection, and the larva of this mosquito was collected from the rice field. Thus, An. nivipes is suspected malaria vector in this basin surrounded by rice field. At present time, the distribution of the malaria vector in Lao PDR is estimated according to the results of a lot of surveys. It is supposed that the existence of An. dirus influence to the high endemic of malaria in the southern provinces. Moreover the biting habit of An. dirus in this area is one of the causes that an impregnated bed net was affected to control malaria.

The vector control using by impregnated bed net (IBN) is one component of the recent global malaria control strategy. It is well known that the health education is important not only to diffuse IBN but also to sustain the project. In the beginning of 2002, the review of Impregnated Treatment Materials (ITM) strategy was carried out in Roll Back Malaria Initiative in Africa. The new strategy consists of both sides between public and private sector in addition to social marketing. In the Mekong region, the ITM programs were carried out using by mainly public sector only, because the peripheral health administration system was well established in comparison with African countries. The other reason, that social marketing was not conducted in Mekong countries, was supposed that the rural areas without suitable media and market were the serious endemic of malaria. When the project is being carried out in the wide area from now on, however, the only public sector is supposed not to be able to sustain the project. In fact, ITM project was expanded to whole country in Lao PDR, the re-dipping of IBN was not carried out in several communities. It is recommended that the joining of private sectors becomes important to sustain the project from now on in Mekong countries.
During about 20 years from 1982 to date, we made an intensive investigation on leishmaniasis in South and Central Americas, especially in Ecuador. The research project was supported at the early phase by JICA, and then supported by the Ministry of Education, Science, Culture and Sports, Japan. Such a long term financial support has been playing the role of driving force to disclose the bio-medical features of New World leishmaniasis. In the project, an intensive study has been done, in order to get information on the disease and its transmission, from multidisciplinary points of view. The aim of project at the early phase was to consider a suitable control measure, by disclosing transmission mechanisms of the disease in each endemic area of Ecuador. By performing country-wide epidemiological surveys, factors relating to the transmission of leishmaniasis, such as the causative agents (6 Leishmania spp.): L.(L.) mexicana, L.(L.) amazonensis, L.(L.) major-like, L.(V.) braziliensis, L.(V.) panamensis, L.(V.) guyanensis, the vector sandflies (4 Lutzomyia spp.): Lu. hartmanni, Lu. trapidoi, Lu. gomezi and Lu. ayacuchensis, and the reservoir hosts (8 spp. of mammals): anteaters, sloths (2 spp.), squirrels (2 spp.), kinkajous, rats and dogs, have been known at different endemic areas of Ecuador. L.(V.) equatorensis from arboreal mammals (sloth and squirrel) was described as a new species, with no human cases. Andean type of leishmaniasis was reported for the first time in Ecuador; the disease form is very similar to Peruvian uta, but the causative agents and vector sandflies are completely different. We tried to search for suitable drugs and treatments of the disease, which would be effective and easily applicable at field conditions; several ointments, lotions and oral drugs such as antimalarials were found to be effective and useful. Furthermore, comparative study was made to have a suitable sampling method applicable for molecular techniques, such as PCR diagnosis. Besides, in the talk several experiences were mentioned, demonstrating cases we face to the problems at field surveys, taking blood samples, skin-testing and etc.
The host immune system is equipped with two arms of immunity at mucosal and systemic compartments. The basic foundation of the immune system was established according to the extensive molecular and cellular characterization of lymphocytes and their associated regulatory molecules in the systemic compartment. To this end, thymus, bone marrow, spleen and peripheral lymph nodes are thought to be the immunological nest of the systemic immune system. In addition to the systemic immunity, the host is equipped with the mucosal immune system providing a first line of defense against numerous foreign antigens which invade the respiratory, intestinal and reproductive tracts. The mucosal immune system has a difficult task: it has to protect approximately 400 m² of mucosal surface area and it must differentiate between beneficial and unwanted antigens at the site of entry.

For providing a first line of defense at such a large mucosal surface, secretory IgA (S-IgA) is a major antibody. For the induction of antigen-specific S-IgA immune response at mucosal surfaces, a unique concept of the common mucosal immune system (CMIS) need to be considered. The CMIS consists of inductive sites (e.g., nasopharyngeal associated lymphoreticular tissue [NALT] and gut associated lymphoreticular tissue [GALT]) and effector sites (e.g., lamina propria and epithelium of the respiratory, intestinal and reproductive tissues). To this end, nasal and oral immunizations have been shown to be effective for the induction of antigen-specific S-IgA response in the distant mucosal effector compartment. Further, mucosal immunization can induce antigen-specific immune responses in the systemic compartment. Thus, the establishment of two layers of the protective immunity can be accomplished by oral or nasal vaccine.

NALT is considered to be an important inductive site for the induction of antigen-specific mucosal and systemic immunity against nasally-administered vaccine antigen. In contrast to Peyer’s patch (PP), an example of GALT, cytokine and corresponding receptor regulated molecular machinery for the tissue organogenesis of NALT remains to be elucidated. Based on the result obtained by the histological and growth kinetic study of normal and specific gene manipulated mice (e.g., IL-7R⁻ and LTα⁻), it was shown that NALT organogenesis is independently operated from a group of known cytokine signaling pathway via IL-7R and LTβR for the generation of secondary lymphoid tissue (e.g., PP).

Our recent investigations have provided new observations that mucosal IgA antibody can be developed in a CMIS-independent manner. IgA committed B-1 cells expressing IL-5R and IL-15R are responsible for the generation of CMIS-independent IgA, while B-2 cells responding to IL-5 and IL-6 are the major source for CMIS-dependent IgA. Taken together, these findings suggested an interesting and new possibility that the mucosal surface in protected by S-IgA generated from the two distinct sources of mucosal B cells.

According to these unique features of the mucosal immune system, our current effort is also focused on the development of novel antigen delivery vehicle for the induction of antigen-specific IgA response. Our recent findings demonstrated that Fusogenic-liposome can effectively deliver antigen to NALT via M cells and epithelial cells. Nasal immunization with Fusogenic-liposome containing vaccine antigen resulted in the induction of prolonged memory type antibody responses. These new findings will extend the understanding of unique feature of the mucosal immune system for the development of mucosal vaccine for the prevention and control of infectious diseases.
Vector borne diseases (VBD) are very important in tropical diseases. The pathological agents of VBD include wide range of organisms such as virus, rickettsia, bacteria, spirochete, protozoa and nematode. Vectors are blood sucking insects such as mosquito, black fly, tsetse fly, sand fly kissing bug and so on, and also ticks and mites. It is difficult to control or to eliminate VBD because the disease are depending on very complicated backgrounds and related deeply to the environments. Vector species and number are numerous and area specific, and easy to develop insecticide resistant.

In the last 30 years of 20th century, molecular biology, molecular genetics and molecular technology have developed very much. Based on these development, some trials to establish strategies for VBD control by molecular technique have been performed. One of the trials is to produce a vector which cannot transmit pathogen by genetic transformation. Technology for producing a transgenic mosquito was established first by transforming EGFP gene by using minos as transposon (Crisanti et al 2000). After some trials, a transgenic mosquito, Anopheles stephensi, which cannot transmit malarial parasite Plasmodium berghei was produced by transforming a synthetic gene of SM-1 (sali-vary gland / midgut adhesive molecule) (Jacobs-Lorena et al., 2002).

The genome of a malarial vector mosquito Anopheles gambiae was published very recently (in Nature, October 2002). So we can find some more candidate genes to transform a vector mosquito, for instance, malarial resistant genes against malarial parasite, and can produce more mosquitoes not to transmit malaria. However, before releasing transgenic mosquitoes we have to develop our technology to replace the natural population to the transgenic populations of mosquitoes and assess the effects to the natural ecosystem.

In the fruit fly Drosophila, female-specific lethal transgenic insects were developed using a sex specific promoter to drive the expression of a repressible transcription factor, which in turn controls the expression of toxic gene product. This principle is applicable to medically important dipteran insect mosquitoes. Using this strategies insect population is more easily controlled than the sterile insect technique.

Some more example trials for developing strategies to control vector borne tropical diseases by studies of molecular and genetic biology of vectors were presented in the lecture.
In the present talk, many great names of history in Japanese Tropical Medicine were mentioned. They were Drs. Yoshio Sawai, Manabu Sasa, Kanzen Teruya, Hachiro Sato, Yoshito Otsuji and others who worked with tropical medicine-related health problems/diseases such as, snake (*Habu*) bite, Tsutsugamushi disease, malaria, filariasis, schistosomiasis, paragonimiasis and other infectious or parasitic diseases. About 40 years ago, all these diseases were highly prevalent at country-wide in Japan and they were great public health problems, because of poor sanitary conditions and insufficient political attention. For example, snake (*Habu*) bite was one of the most serious health problem in subtropical regions of Japan, especially in Okinawa and Amami-Oshima islands. During 13 years from 1959 to 1971, 3,510 snake bite cases were reported from Amami-Oshima island; the average fatality rate was 0.8% in the area. Dr. Yoshio Sawai, a microbiologist at the Institute of Medical Science at the University of Tokyo was the person who hardly worked with the *Habu* bite. In 1952 he was appointed as Associate Professor and chief of the newly-established laboratory of biological products at the Institute. Antivenin productions including these for *Habu* venom was one of the traditional work in the institute. Naturally Dr. Sawai tried to develop more effective and useful *Habu* toxoid. He frequently visited Naze Health Center, Amami-Oshima island and observed there many patients suffered from serious and aggressive *Habu* bites with severe necrosis at the site of snake bite. He started laboratory investigations to improve the treatment of *Habu* bite, together with his co-workers at the Institute. Their purified and lyophilized anti-venin was favorable to decrease numbers of the severe cases of *Habu* bites when applied clinically at Amami-Oshima island. However, yearly occurrence of severe clinical cases were still found in the area, in spite of such a Dr. Sawai’s untiring efforts. After analyzing many clinical cases of *Habu* bites, Dr. Sawai reached to the conclusion that there might be a limitation in their serum treatment against the snake bite, because the development of local lesions by the large amount of *Habu* venom was too rapid for the anti-venin treatment to prevent. Then, in 1963, Dr. Sawai reported a dihydrothioctic acid mixed with different concentrations of the *Habu* venom; the preparation inhibited lethal effect of venom when tested in experimental animals such as mice, rabbits and guinea pigs. In April, 1965, the first large scale trial of active immunization using this product was undertaken in persons living at remote areas with high-risk of *Habu* bites. In Amami-Oshima and Okinawa islands, 43,446 persons received the *Habu* venom toxoid during 3 years of active immunization from 1965 to 1967. Statistic analysis of these trials showed that the venom toxoid used was effective, enough to reduce the rate of necrotic cases by 50%. The improvement of the *Habu* toxoid developed by Dr. Sawai’s group has been promoted by the Committee sponsored by the Ministry of Health and Welfare in Japan. Later, Dr. Sawai expanded his research to Southeast Asia and India, also contributing greatly to this world-wide life threatening snake bites; he is 90 years old, living in Ohta city, Gunma, Japan. Other works by the great names mentioned above were also discussed briefly in the session, showing many slides.
Symposium

S - I - 1) CURRENT STATUS OF EMERGING PROTOZOAL INFECTIOUS DISEASES: CRYPTOSPORIDIOSIS, CYCLOSPORIASIS, AND MICROSPORIDIOSIS

MOTOHIRO ISEKI

Department of Parasitology, Graduate School of Medical Science, Kanazawa University

Cryptosporidiosis, caused by Cryptosporidium parvum infection, is recognized as acute, self-limiting, gastroenteritis in immunocompetent humans and persistent and potentially fatal in immunocompromised subjects, particularly those with AIDS. The number of the infection is estimated to be 250 millions to 500 millions every year, in both developed and developing countries. Recently, reports of imported cases among travelers to less developed countries, domestic infections among both immunocompetent or immunocompromised hosts, and outbreaks associated with public water supply are increasing in Japan. Diagnostic methods for demonstrating the oocyst or antigens in clinical specimens are well developed. While, we still have no consistently effective chemotherapeutic agent for use. Development of effective drugs for treatment and of control measures for waterborne outbreaks is urgently needed.

Cyclosporiasis is caused by Cyclospora cayetanensis infection. The organism is a newly recognized coccidian parasite inhabiting small intestinal epithelial cells and causes prolonged watery diarrhea, fatigue, and anorexia in humans was reported at least as early as 1979, it was initially called a coccidian-like body or a cyanobacterium-like body (CLB). In 1993, the organism was identified as a protozoan of the genus Cyclospora, and the species name of C. cayetanensis was proposed in 1994. The disease is widely distributed in tropical and subtropical countries. Since 1996, more than 10 cases among travelers to developing countries and a case suspected to be infected by ingesting imported fresh vegetables have been reported in Japan. Symptomatic infections can be treated with trimethoprim-sulfamethoxazole.

Microsporidiosis, caused by infection with several species of the phylum Microspora, is an important opportunistic infectious disease among AIDS patients and appears to have worldwide distribution. At present, 13 species of microsporidia have been identified as potential pathogens in humans. The first report of diarrheal syndromes associated with microsporidiosis and HIV infection was published in 1985, and the number of case reports increased rapidly after 1990. Chronic diarrheas caused by Enterocytozoon bieneusi or Encephalitozoon intestinalis infection are frequently observed among AIDS patients. While, Enc. hellem and Enc. cuniculi cause keratoconjunctivitis and disseminated infection, Pleistophora spp. and Nosema sp. cause myositis. Recently, cases of microsporidiosis have been reported in Japan. Microscopic detection of the parasite requires special staining methods and adequate microscopic techniques. Although two agents, fumagillin and albendazole, have been reported to have activity against microsporidia both in vitro and in vivo, treatment efficacy is poor except in the case of infections caused by Encephalitozoonidae.

S - I - 2) CRYPTOSPORIDIOSIS AND CYCLOSPORIASIS: THE CLINICAL FEATURES AND OCCURRENCE IN JAPAN

GOHTA MASUDA

President, Tokyo Metropolitan Kiyose Children’s Hospital

Cryptosporidiosis

Cryptosporidium sp. (mainly C. parvum) causes transient non-bloody diarrhea in normal hosts, while this pathogen produces severe, intractable, and recurrent non-bloody diarrhea, and in some cases biliary tract and/or respiratory tract infections in immunodeficient hosts. Gut infections with this pathogen tend to demonstrate various degrees of diarrhea, abdominal cramps, flatulence, general fatigue, nausea and vomiting. Many sporadic diarrheal cases due to this protozoa are thought to exist in Japan, however, when such patients seek medical care, they are often misdiagnosed to have culture-negative and antimicrobial-refractory diarrhea.
Large waterborne outbreaks of diarrheal illness due to this pathogen have been reported in Japan involving 461 patients (in Kanagawa-ken, 1994), 8,812 patients (in Saitama-ken, 1996), 129 and 170 patients (in Niseko and Toyako lake side in Hokkaido, respectively, in 2000).

From a prospective study performed at Tokyo Metropolitan Komagome Hospital from 1990 to 2000, Cryptosporidium oocysts were positively detected in 18 out of 508 specimens (3.5%) from diarrheal patients who had recently traveled overseas, in none of 605 specimens (0%) with no history of overseas travel, and in 4 out of 140 specimens (2.9%) from HIV infected patients.

Cyclosporiasis

Cyclospora cayetanensis causes non-bloody watery diarrhea which clinically resembles cryptosporidial diarrhea except for the fact that diarrhea with C. cayetanensis tends to be recurring. The administration of sulfamethoxazole/trimethoprim is usually effective for this kind of diarrhea. Cyclosporiasis in immunodeficient patients tends to lead to diarrhea with longer duration than that observed in normal hosts, and this infection is usually non-fatal even in these patients.

A few sporadic cases of Cyclospora enteritis have been reported in Japan. A study based on 1,071 stool specimens observed from patients at Tokyo Metropolitan Komagome Hospital from 1996 to 2001 indicated the positive rate of this protozoa among diarrheal illness to be 0.7% (3 out of 410 stool specimens were positive) in patients with a recent history of overseas travel, none (0/513 specimens) in indigenous diarrheal patients, and 0.7% (1/148 specimens) in HIV-infected patients.

S - I - 3) EMERGING AND RE-EMERGING ARBOVIRAL INFECTIOUS DISEASES

TOMOHIKO TAKASAKI and ICHIRO KURANE

Department of Virology 1, National Institute of Infectious Diseases

Arboviruses are one of the major pathogens of emerging and re-emerging infectious diseases. West Nile fever (WNV) epidemics since 1999 in the United States and dengue outbreak in Hawaii during 2001-2002 are valuable lessons. Global warming is a favorable condition for insects such as mosquitoes and ticks. We need to pay more attention to emerging and re-emerging arboviral infectious diseases in Japan as well as in other areas of the world.

Key words: West Nile virus, dengue virus, imported case

S - I - 4) CURRENT STATUS OF EMERGING AND RE-EMERGING BACTERIAL INFECTIOUS DISEASES

TSUYOSHI NAGATAKE

S - II - 1) DETECTION OF NEW ENDEMIC AREAS OF CUTANEOUS LEISHMANIASIS IN PAKISTAN: A SIX-YEAR STUDY

ABDUL MANAN BHUTTO¹, RASHID AHMED SOOMRO², SHIGE幽 NONAKA² and YOSHIHISA HASHIGUCHI¹

1. Department of Dermatology, Chandka Medical College/Hospital Larkana, Sindh, Pakistan
2. Department of Dermatology, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan
3. Department of Parasitology, Kochi Medical School, Nankoku, Kochi, Japan

Cutaneous Leishmaniasis (CL) is endemic in Pakistan and is widely spreading. We report some new endemic areas of CL in the country. A total of 1210 cases of CL visited our department from 1996 to 2001. Among them, 760 were the residents of Jacobabad, Larkana and Dadu districts of Sindh province and had never been traveled to the endemic
areas before. These districts have never been reported/recognized as endemic for CL. Others were the residents of endemic areas of Balochistan province. All the patients were between 2 and half months and 65 years of age. Three hundred and ninety two patients were females and 368 were males. Duration of the disease ranged from 2 months to one and half year. Most of the patients had single lesion on the face and/or extremities. Clinically the disease was classified as dry popular type, 407 cases; dry ulcerative type, 335 cases; and wet ulcerative type, 18 cases. No any case of muco-cutaneous or visceral leishmaniasis was found during this period. Diagnosis was made on clinical presentation; Giemsa stained smear test; histopathological; and polymerase chain reaction (PCR) results. Smear test was positive in 845 cases, while 365 cases were histopathologically positive. Ultrastructural study was performed using the specimens of few cases. Leishmania parasites were detected in the dermal tissues as well as macrophages. About 200 cases were studied by PCR, among them 92% were positive for Leishmania parasite. All the cases were treated with the meglumine antimonate 600 mg/day in adults and 15 mg/kg/day in children as intramuscularly for 20 consecutive days.

On the basis of our findings we propose that Jacobabad, Larkana and Dadu districts could be considered endemic for CL. Wet and dry type lesions indicates the presence of both the L. (Leishmania) tropica and L. (L.) major in this tropical region. This will be confirmed by sequencing technique in future.

S - II - 2) CURRENT STATUS OF LEISHMANIASIS IN ECUADOR

MANUEL CALVOPINA and YOSHIHISA HASHIGUCHI

Department of Parasitology, Kochi Medical School, Nankoku-shi, Kochi 783-8505, Japan

The leishmaniasis is endemic throughout Ecuador and the incidence is increasing annually. Cases had been reported from all geographical regions mainly in populations living in rural areas. Recent studies found that the disease is wide spread in most provinces and is considered a health problem in the country. National data showed that the rate of cases increased from 4.36 in 1998 to 20.24 in year 2001 per 100000 inhabitants.

The localised cutaneous form (LCL) represents more than 95.0% of the total cases. The common LCL form is ulcer, but non-ulcerated forms such as papulas, plaques and nodules are also seen. Diffuse cutaneous form (DCL) is very rare. Mucocutaneous form (MCL) had only been reported from the Amazonian lowlands. In an active survey we found 13 cases of MCL in the region. A confirmed case of DCL was reported to be produced by L. (L.) mexicana.

Using molecular techniques, seven species of the genus Leishmania has been identified from the Pacific side: L. (V) braziliensis, panamensis, guyanensis, equatorensis, L. (L.) amazonensis, mexicana, and L. (L.) major-like; two from the Andes: L. (L.) mexicana and L. (L.) major-like and two from the Amazon: L. (V) braziliensis and L. (L) lainsoni. Confirmed vectors of Leishmania in the Pacific side are Lutzomyia trampidoi, hartmanni and gomezi. More recently Dujardin demonstrate cryptic species in Lu. trapidoi. In the Andean foci Lu. ayacuchensis has been incriminated as a vector. In the Amazonian no vector has been found infected, though there are several man-biting species Lu. serrana, Lu. nevesi, etc. Non-human hosts of Leishmania detected to date are sloths, squirrels, anteaters, rats, kinkajous and canines. Dogs are recorded as hosts of Andean L. (L.) mexicana.

In most endemic areas, leishmaniasis is well known and patients use several “folk methods” for treating it. The drug used is Glucantime provided by the government free of charge. Other drugs such as mefloquine, artesunate, allopurinol, paromomycin and fosfomycin had been tested in small trials with variable efficacy.

Current control measures rely on chemotherapy to alleviate the disease. No vector control has been implemented. Extensive vaccination trial has demonstrated that a cocktail of five killed Leishmania induces significant protection. A new phase III study (Biobras) with killed promastigotes+BCG, is in final step and under revision by TDR/WHO.
Leishmaniasis is caused by parasites belonging to genus *Leishmania*. Human infections display a spectrum of manifestations ranging from cutaneous, mucocutaneous to generalized systemic visceral disease. The genus *Leishmania* is taxonomically classified into subgenus *Leishmania* and subgenus *Viannia*. The parasites reside in different regions of the digestive organ of sandfly, *Phlebotomus* (in the Old World) and *Lutzomyia* (in the New World), and their classification is yet to be completed. The cytochrome b gene (*Cyt b*) is contained in the mitochondrial genome and encodes an enzyme in the respiratory chain of mitochondria. The *Cyt b* gene has been used for phylogenetic studies for animals, plants and fungi. Here, we report determination of complete nucleotide sequences of the *Cyt b* gene in parasites belonging to the genus *Leishmania* (14 strains) and those belonging to the genus *Endtrypanum* (4 strains). Phylogenetic analysis of these parasites, based on the difference of *Cyt b* gene sequence, agreed well with the previous classification of above three groups. The difference was consistent with different geographic distribution of parasites and with different clinical manifestations associated with parasites. In addition, we present recent progress in our gene expression profile analysis of *Leishmania*-infected macrophages. We show that expression of the genes encoding MCP (macrophage chemoattractant protein)-5 and STAT (signal transducer and activator of transcription)-1 are up-regulated during infection as examined by the quantitative real-time RT-PCR analysis.

S - II - 4) ADVANCE IN DEVELOPMENT OF ANTI-LEISHMANIAL DRUGS AND RESEARCH ON MECHANISMS OF DRUG RESISTANCE IN *LEISHMANIA*

KEN KATAKURA¹, MAMORU SUZUKI², KOJI YOKOYAMA³, MAKOTO MIYAJI⁴, KAZUKO NISHIMURA⁵, OSAMU KANEKO⁶, MOTOMI TORII⁷, HIROSHI FUJISE⁸, YOSHIHISA HASHIGUCHI⁹

¹Department of Parasitology, Gunma University School of Medicine,
²Research Center for Pathogenic Fungi and Microbial Toxicseses, Chiba University,
³Department of Parasitology, Ehime University School of Medicine,
⁴Laboratory of Pathophysiology, School of Veterinary Medicine, Azabu University,
⁵Department of Parasitology, Kochi Medical School

Pentavalent antimonial drugs have been used for treatment of leishmaniasis since 1940’s. Antimonial drugs, however, require to be injected and produce side effects, thereby oral safety anti-leishmanial drugs have long been desired. Sterol biosynthesis pathway in *Leishmania* appears to be very similar to that in yeast. Thus, oral antifungal drugs have also been used for chemotherapy of leishmaniasis. We found that SS750, a synthesized new triazol anti-fungal compound, was effective on both cutaneous and visceral leishmaniasis in mice. Skin lesions of mice, which orally received SS750, were smaller than those of untreated control mice infected with *Leishmania amazonensis*. Parasite burden in the liver was low in mice orally treated with SS750 than that in control mice infected with *L. donovani*. These results suggest that SS750 may be applicable for treatment of various types of leishmaniasis, although miltefosine (hexadecylphosphocholine) is now the most hopeful oral drug for treatment of visceral leishmaniasis.

Studies of drug resistance mechanisms in *Leishmania* is necessary for more rational use of drugs and drug combinations to minimize or circumvent resistance, since antimony resistant *Leishmania* species are found in endemic areas, especially in India. Studies on multidrug resistance in mammalian cancer cells lead the discovery of the ATP-binding cassette (ABC) proteins, which form one of the largest protein families and its members are present each
kind of organism from bacteria to human. Among the ABC proteins, MDR1 (multidrug transporter or P-glycoprotein) and MRP1 are most involved in drug resistance in human. MDR1 is localized in the plasma membrane and can extrude a wide range of structurally unrelated hydrophobic toxic compounds from the cell. MRP1 transports both hydrophobic anticancer agents and glutathione conjugates. In Leishmania, currently two ABC transporters can mediate drug resistance in experimental conditions. PGPA, a member of MPR subfamily, appears to transport antimony- and arsenite-thiol conjugates into intracellular vesicles. Leishmanial MDR1, a homologue of mammalian MDR1, can confer resistance to a number of anticancer drugs of the mammalian MDR1 spectrum and appears to be localized in the multivesicular tubules of lysosomes. We have cloned and characterized a new member of ABC transporter, LaMDR2 in L. amazonensis, which can confer resistance to 5-fluorouracil by probably exocytosis. These results suggest that drug resistance mediated by ABC proteins in Leishmania is related to secretory and exocytotic pathway of the parasites.

S - III - 0) FOR THE FRUITS OF TROPICAL MEDICINE

SINJIRO HAMANO

Department of Parasitology, Faculty of Medical Sciences, Kyusyu Univ.

The tropics are inevitably hotbeds of pathogen transmission because of its various natural and socio-economical conditions, where there are all of the disease transmission structures including pathogens, vectors and diseases, which investigators have been pursuing. The final goal of “Tropical Medicine” is “To prevent, diagnose and treat diseases be there, mainly composed of infectious disease”, and “Tropical Medicine” is a scientific field containing all fields concerning it. For the fruits of “Tropical Medicine”, in other words, in order to contribute to the prevention, the diagnosis and the treatment of diseases there, what should we do in future?

In this session, young scientists will introduce their works in the past and now at first, and then survey a future concerning the study. Furthermore, for the fruits of “Tropical Medicine”, we would like to discuss about

1. What are deficient in?
2. How can we get the deficient one (knowledge, technique, skill, idea, personal connections, language ability, funds, a position, fields etc.)?
3. What kind of strategies we can map out to survive in each organ while pursuing Tropical Medicine?, as an individual investigator or as an organization, Japanese Society of Tropical Medicine.

I hope that this session will encourage young scientists in making a dream come true.

S - III - 1) SYNTHESIS OF USEFUL ANTIBODY MOLECULES USING THE COMBINATORIAL LIBRARY AND THE PHAGE DISPLAY TECHNOLOGIES

TETSU YAMASHIRO

Department of Infectious Diseases, Oita Medical University

DEN infection has been a big concern in a DEN endemic society for decades all over the world. According to the investigations conducted by the WHO, the worldwide incidence of dengue fever (DF) is estimated from twenty-five million to fifty million. An incidence of the Dengue shock syndrome (DSS), an atypical and much more serious response to the viruses characterized by hemorrhage is considered more than five hundred thousand in the whole world.

Recently, we have constructed a phage library with material of DEN hyper-immune higher primate and from which a panel of Fab specific to DEN virions were isolated. Biological analysis of those Fabs were performed and described. Those Fabs obtained through a combination of combinatorial library and phage display technologies
should be valuable for use in passive prophylaxis or therapy for humans against dengue infection.

When one attempts a collaborating study with an institute of distinct culture, attention should be paid on the following points. Choose a theme appropriate to demands of the society where the study would be conducted. Have the clear objective. Prepare the detailed work plan. Share detailed information among the study team. Make an effort to reduce mistake-prone steps. Design and perform a pilot study in order to obtain information needed for refining the study. Distribute the results and interpretation obtained from the study to each person or institute involved. Careful consideration should be paid to the human rights when collecting and handling human samples.

We are aiming at the preparation of human Fabs possessing anti-dengue neutralization activity through the combinatorial science. Is it possible to construct an Fab library using human material obtained from healthy volunteers who had been recovered from DEN infection after obtaining full consent to participation in a study in DEN endemic area? The constructed library should contain higher population of phage clones displaying Fab specifically neutralize DEN and has an obvious advantage in isolation of DEN neutralizing Fabs over other libraries.

Currently, a large number of epidemiological surveys as well as sample collections for determining pathogens or pathogenesis of microorganisms have been conducted in the field of tropical medicine, however, it also possess a potential dimension for supplying useful molecules for prophylaxis or therapeutic applications against certain infectious diseases.

S - III - 2) MOLECULAR MECHANISM OF MALARIAL SPOROZOITES INFECTION INTO THE MAMMALIAN LIVER

TOMOKO ISHINO, MASAO YUDA, KAZUHIKO YANO, YASUOCHINZEI

Department of Medical Zoology, School of medicine, Mie University

Even today, malaria is one of the most devastating infectious diseases, and it kills more than two million people in the world at a year. The emergence of resistant parasites makes it more difficult to control this disease. Moreover, preventive method, such as vaccine, has never developed yet.

Malarial sporozoites injected by mosquito into mammalian host rapidly enter circulatory system, then infect hepatocytes, where they develop to thousands of merozoites. Therefore sporozoite infection of hepatocytes is the obligate step for malaria transmission to mammalian host. However its molecular mechanism is unclear yet. Aimed at elucidation the molecular basis of parasites infection, we applied a reverse genetial method, as ‘post genome study’.

As it was reproted that infectivity to the liver was acquired after sporozoite invaded the mosquito salivary gland, we constructed EST database of P. berghei sporozoites collected from salivary gland. Then, by gene targeting method, function of some proteins expressed in salivary glands sporozoite were analyzed. As focused on parasite-host interactions, putative membrane associated or secretory proteins were selected preferentially. Here we report that a salivary gland sporozoite specific protein was identified and it involved in parasites’ infectivity of the mammalian liver.

By the way, it was known that administration of radiation attenuated sporozoites induces preventive immunity against malaria. Thus it was believed that some sporozoite proteins should be good targets for developing malarial vaccine. Our strategy will facilitate on understanding of the molecular basis of malarial of vertebrate hosts, and may lead to the development of vaccines against malarial transmission.
S - III - 3) INVOLVING TROPICAL MEDICINE PRACTICALLY

TOSHIKI AWAZAWA
The Research & Control of Infectious & Prasitic Diseases Project,
Kenya Medical Research Institute/JICA

The presentation focused on two areas.
The first outlined the various approaches in participating international health in the tropical regions. Having learnt from personal experiences, from working as a staff member in the health service providing facility such as a hospital, to a project co-ordinator in the ministry of health, the disease control programme onto the existing health infrastructures seems feasible approach. One of these infrastructures is the school system. Schools have increasingly been recognized as one of the most convenient and logical springboards from where to promote healthy behaviours and to deliver certain health services. In the context of de-worming campaigns, schools offer one of the most efficient and effective channels to one of the most vulnerable groups. Moreover, the ability of non-medical personnel to deliver the drugs means that the number of children and the speed at which they can be reached is massively accelerated.

The second part of the presentation focused on the parasite control activities at Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC) of the Kenya Medical Research Institute. ESACIPAC is one of the three centres of the Global Prasite Control Initiative, also referred to as the Hashimoto Initiative. Its mandate is to coordinate capacity building for parasitic diseases control activities in the regional countries, namely, Kenya, Uganda, Tanzania/Zanzibar, Malawi, Zambia, Botswana and Zimbabwe. Its primary functions include training of personnel and networking among them. ESACIPAC will involve implementation of school based parasite control activities in Kenya. The strategy for coordinating effort of all other technical co-operation project funded by JICA (Japan International Co-operation Agency) is also discussed.

S - III - 4) WHAT IS THE TROPICAL MEDICINE IN JAPAN? - A QUESTION FROM A YOUNG SCIENTIST -

OSAMU KANEKO
Department of Molecular Parasitology, Ehime University School of Medicine

So-called “Tropical medicine” consists of two research areas. First area pursuits to study a basic biological aspect of the tropical diseases and develops tools to prevent and/or treat human beings from diseases by biomedical research. The other area pursuits to study a public health system to develop strategies to apply tools produced. These two areas simultaneously contribute to solve the tropical health problem. Increasing funding in the basic research area of the infectious diseases has stimulated the activity of this area and the number of young researchers has increased in the past 10 years. Furthermore, based on the Hashimoto Initiative, projects aiming human resource development and information networking to control parasites in Asia and Africa were launched with the strong supports by JICA. However, despite of these situations, there is a big gap between the basic research area and the disease control area. One of the reasons of this gap can be explained by the limited human resources of this area, especially in the research area conducted in the tropical field. Improved health situation in Japan has reduced not only infectious diseases, but also researchers in this area. As mentioned at the beginning, successful control of the diseases requires tools and strategies, thus our current need is to promote the authentic epidemiology in order to create better strategies to combat tropical diseases. Here I propose 3 activities, 1) Promoting more field studies in the tropical countries to stimulate the academic side of this area; 2) promoting young researchers in this area and recruit researchers from other areas to increase the population of this area; and 3) encouraging to attend international meetings of tropical medicine to upgrade the quality of this area. These activities would produce fruitful outcomes from Japanese Society of Tropical Medicine in the next 20 years.
ADEQUATE TREATMENT AND EARLY DIAGNOSIS OF LEPROSY

ATSUSHI HOSOKAWA, MOHAMMED A.K.KHAN, SHIGEO NONAKA

Department of Dermatology, Faculty of Medicine, University of the Ryukyus

In fact, a large number of patients with leprosy rapidly decreased by multi-drug-therapy (MDT). But newly detected patients with leprosy has been increased in number in the endemic areas, especially in India, Brazil and Southeast Asia etc. Three important measures of leprosy control are to be discussed, namely chemotherapy, early detection in the early stage of leprosy and the prejudice for the disease.

1) Chemotherapy; Four leprosy cases with sequelae caused by neuritis which increased after starting MDT and two cases without sequelae were shown. For a patient with borderline group leprosy, who showed severe neuritis and deformity of the fingers after starting of the medication, the chemotherapy was considered to be inadequate. A patient with an annular erythema on the on left buttock, diagnosed as to be tuberculoid leprosy, received a modified MDT, comprising of 450mg of RFP per monthly and 75mg of DDS for a month, healed completely within a few months. Two contrastive patients with borderline group leprosy showed similar infiltrated erythema on the right face were reported. In the course of chemotherapy, the deformity of face became obvious and flacid palsy of the right orbicularis oculi muscle appeared in a borderline tuberculoid-middle borderline leprosy patient. On the other hand, another patient with borderline tuberculoid leprosy healed without any sequelae. As shown in the above mentioned cases, each of them showed different and various responses to chemotherapy during the course of the treatments. Therefore the simplified chemotherapy for the simplified classification considering sensory loss, neuritis and the site of lesion are need-ed to prevent the sequelae. For example, n1 indicate the type of leprosy with sensory loss and minor neuralgie, n2 indicate the type of leprosy with sensory loss and severe neuralgie, h1 indicate the site of lesion at the axillary, inguinal, perineal lesion, buttock and scalp, h2 indicate site of lesion at the hands, feet-leg, face and ear.

2) Early detection; Indeterminate leprosy case healed completely in a few months. Therefore, it is very important for elimination of leprosy to detect leprosy patients in their early stage of the disease. For this purpose, paramedical and non-medical staffs may play a great role.

3) Prejudice; Though the prevalence rate of leprosy in Japan became below 1.0 per 10.000 approximately 30 years ago, prejudice for the disease is still remain especially in old people.

Japanese medical personnels can assist to resolve the above mentioned problems and shortening the period for elimination or eradication of the dis-ease in the endemic areas.

AROUND THE FUNDAMENTAL LEPROSY RESEARCH

- HOW TO PROCEED THE RESEARCH OF A CLASSICAL INFECTIOUS DISEASE IN GENOME ERA -

SHIN SASAKI

Department of Bioregulation, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Leprosy is a classical infectious disease caused by Mycobacterium leprae, which was discovered by G. A. Hansen in 1873. The disease has been documented since antiquity and still continues to be endemic in some developing countries. M. leprae is an exceptional bacterium because of its long generation time and no growth in artificial media. Entire sequencing of the bacterial genome revealed numerous pseudogenes which might be responsible for the very limited metabolic activity of M. leprae. The establishment of effective combination chemotherapies in 1970s prove a great boon to the victim of leprosy. Multiple drug therapy (MDT), which consists of several antibiotics linked to simplified diagnostic regimen, has become increasingly popular in the countries where the disease is endemic. A significant drop of the disease prevalence has been achieved worldwide, and the endemicity of leprosy remains in few countries. To achieve the world without leprosy, we have many questions to be answered. How the bacteria are disseminated?
Why intrafamilial infection is likely? Is there any source of infection other than symptomatic patients? Moreover, no diagnostic marker nor vaccines are available. A compendium for the current leprosy research is provided and the future prospects are also discussed.

S - IV - 3) PRESENT SITUATION OF HANSEN’S DISEASE IN THE WORLD

NORIHISA ISHII

Department of Bioregulation, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Leprosy patients in Japan was reported that about 30,000 in 1900, which decreased to about 16,000 by 1919. From roughly 1955, due to the improvement of public health and appearance of remedies, new patients decreased rapidly. Recently, there are less than 10 new patients per year. On the other hand, non-Japanese patients (imported cases) have been on the increases since roughly 1991, counting about 10 patients per year. It is, therefore, anticipated that there will be no leprosy patients among Japanese in near future. Non-Japanese patients, however, appear among a large number of employees aged 20s~30s from Brazil and the Philippines where leprosy is still endemic.

Global situation owing to multidrug therapy (MDT) pursued by WHO; more than 10 million leprosy patients worldwide were cured from 1985 to the end of 2001. The registered cases decreased to 635,404 by the beginning of 2002 and the prevalence was 1.0 per 10,000 population, which is a 88% reduction from 1985. After treatment for 6 months in PB cases and for one year in MB cases, they are regarded as cured and withdrawn from the WHO. The relapse rate is about 0.1% per year. Only few MDT-resistant cases have been found. Physical disability may be seen after peripheral nervous impairment (two to three million cases), and many have difficulty returning to and attaining social lives. In spite of the promotion of MDT by WHO, as many as 760,000 new patients are still registered every year, and that is presumably due to improved diagnosis and enhanced surveillance for many new cases including ones overlooked in the past. The countries with many yearly registered new leprosy patients during the year 2001 are; India (617,993), Brazil (41,070), Indonesia (13,286), Bangladesh (10,740), Myanmar (9,684), and Nepal (13,830).

Full control of leprosy has eluded mainly in Angola, Brazil, India, Madagascar, Mozambique, Myanmar and Nepal. These countries are committed to stepping up leprosy control activities. Access to information, diagnosis and treatment with MDT is essential. Information campaigns about leprosy in high risk areas are crucial so that patients and their families, who were historically ostracized from their communities, are encouraged to come forward and receive treatment. Today, diagnosis and treatment of leprosy is easy. Essential work is being carried out to integrate leprosy services into existing, general health services. This is especially important for communities at risk for leprosy, which are often the poorest of the poor and under-served.

S - IV - 4) PRESENT SITUATION OF LEPROSY DISEASE IN MYANMAR

TIN AUNG SOE {M. B; B. S}, Team Leader

Leprosy Control Programme, Myanmar (Leprosy Research Center, NIID, Japan)

The Union of Myanmar is one of the hyper endemic countries with leprosy disease. Although the leprosy disease burden has significantly reduced to a low level in Myanmar, leprosy elimination activities need to intensify to achieve the elimination goal in time. National Leprosy Elimination Programme has been already committed to reach the elimination at the national level by reducing the prevalence below one case per 10,000 populations by the end of year 2003. To achieve the elimination of leprosy, the programme was fully integrated in Basic Health Services. It was decided in 1969 to try a gradual handling over of responsibilities of the vertical leprosy control services to the basic health services personnel. In this presentation I present “the present situation of leprosy disease in Myanmar”
such as disease burden, integration, detection of new cases, prevalence and detection ratio, routine, activities, community awareness and monitoring and supervision. And I also present “cooperation of Myanmar and Japan (JICA) regarding the leprosy disease”. With the cooperation of other organizations such as WHO, JICA and NGOs, we could accelerate the elimination activities towards the goal and also other aspects regarding with leprosy disease. There is a period very near to elimination of leprosy in Myanmar. We conduct the acceleration and sustainability of leprosy elimination activities. The application of multi drug therapy (MDT) is progressing rapidly towards the goal of elimination of leprosy as a public health problem. Preparatory activities for post-elimination strategies are also considered including prevention of disability, disease surveillance, control and prevention tools and etc.

S - IV - 5) ACTIVITES OF JICA PROJECT FOR LEPROSY CONTROL AND BASIC HEALTH SERVICES IN MYANMAR

EJI NAGAO

National Leprosarium Osima Seisho-en

JICA project team has carried out many kinds of provisions against National Programme for Leprosy Elimination in Myanmar, from 2000.

We have conducted training courses of midwives, laboratory technicians, doctors, physiotherapists, nurses, prosthesis therapists, shoemakers, and persons affected leprosy (PALS).

We recommend (1) that special actions for new case detection should be continued, until true elimination on townships and villages level is achieved, (2) that various kinds of useful training should be continued, and (3) that various kinds of effective enlightenments should be held in communities, because leprosy is a chronic disease with extremely long incubation term and PALS with disabilities need many kinds of aids.

S - V - 1) ROLE OF THE JICA ZAMBIA PROJECT IN AIDS CONTROL IN SOUTHERN AFRICA

HIROSHI TERUNUMA

Department of Microbiology, Faculty of Medicine, University of Yamanashi

The AIDS epidemics is still one of the biggest problems in global health issues. At the end of 2002, over 60 million people were infected with HIV. Forty million are still living with HIV and 3 million died during the year 2002. More than 90% of these infected people are living in developing countries and got infected with HIV through heterosexual contact. Zambia has a high seroprevalence of HIV-1. One forth of the adult population in Zambia is infected with HIV. For the past 10 years education has been emphasized to control the spread of HIV in Zambia. While education is still very important, it is, however, not enough to control the HIV epidemic because the number of infected people is still increasing. Further more over 1 million Zambians are living with HIV.

From 1989 to 2000 JICA Zambia infectious disease and infectious diseases control programs established the University Teaching Hospital (UTH) Virology Laboratory to examine viral diseases such as polio, measles, influenza and HIV. For HIV, we introduced flow cytometry and PCR technology at the laboratory in Zambia. In 2001 the JICA HIV/AIDS and TB control project was started in Zambia and this project is contributing to the control of the HIV epidemic. Currently the Virology Laboratory is the only laboratory which can evaluate quality assurance of HIV serological testing and monitoring the condition of HIV-infected patients.

To control HIV/AIDS, this program is closely working with other working groups such as the national AIDS secretariat, voluntary counseling and testing and care program (VCT), prevention of mother to child transmission program (PMTCT), and treatment and vaccine program. The Virology laboratory is currently conducting training and quality
assurance of HIV serological testing for technologists at VCT sites, monitoring of drug-resistant HIV-1 and HIV-1 strain surveillance for vaccine development.

I hope through this program many young Zambian and Japanese experts will gain experiences to fight infectious diseases and will develop better strategies to control HIV/AIDS.

S - V - 2) TUBERCULOSIS CONTROL IN NEPAL AND JAPANESE MEDICAL COOPERATION

KATSUNORI OSGA
Research Institute of Tuberculosis Japan Anti-Tuberculosis Association

In the early 1960s, a Japanese doctor arrived in the Kingdom of Nepal. His name was Noboru Iwamura. As a mission doctor, he worked in the mountainous outskirts of the Himalayas, visiting villages searching for tuberculosis patients. Many locals still remember Dr. Iwamura, one of the most well known Japanese names in Nepal to date. With widespread poverty, however, tuberculosis remained the biggest threat to public health in Nepal.

Following pioneers from the private sector, the Japanese Government started to provide assistance to control tuberculosis in the 1970s. The National Tuberculosis Center was constructed with the Grant Aid scheme in the 1980s. Today, this center functions not only as the headquarters of the National Tuberculosis Program, but also as the regional TB center for South Asia (SAARC TB Center). Coupled with the construction of the TB center, a technical cooperation project by JICA was also carried out. Short course chemotherapy using Rifampicin, which was uncommon in developing countries at that time, was also tried as a pilot study. Technical expertise was provided from the Research Institute of Tuberculosis (RIT), Tokyo. Apart from Japan, no major donors or international agencies provided substantial assistance for TB control in Nepal during this period.

It was in the mid 1990s, when the international arena began to focus on TB control. WHO launched the new strategy called “DOTS” and the World Bank supported its cost-effectiveness. Since then, many new donor partners, such as Norway, UK, and WHO, joined the effort to combat TB in Nepal. With multiple partners providing assistance to the Government of Nepal in different schemes, misunderstanding and friction sometimes occurred. The importance of coordination among donors and UN agencies was recognized.

Today, nearly 90% of the population in Nepal has access to DOTS, with over 85% treated successfully. Japanese technical cooperation in Nepal now has broader aspects. The current JICA project is focusing on “lung health”, targeting childhood ARI and smoking cessation, as well as TB control in difficult population groups and areas. Every year, medical doctors and paramedics from Nepal are trained at RIT; more than 70 Nepalese have been trained in the past 40 years. It is now well recognized that Japanese technical cooperation, both through governmental and non-governmental sectors, has made a significant contribution to human resource development and appropriate technology transfer for TB control in Nepal. However, the approaching HIV epidemic, spreading poverty, and social instability in recent years, all pose a major threat to TB control in Nepal. Long-term collaboration appears necessary to sustain the fight against tuberculosis in Nepal.
3) PROBLEMS OF IMPLEMENTING THE MALARIA CONTROL PROJECT

HIROJI KANBARA

Department of Protozoology, Institute of Tropical Medicine, Nagasaki University

We, the Institute of Tropical Medicine, Nagasaki University have began the JICA partnership project “Malaria control at Lombok and Sumbawa islande in Indonesia” since November, 2001. Since activities necessary for the project included various fields, we, first, formed necessary sections and appointed a responsible person in each section as follows. 1) Section for malaria parasite survey and control. The section was devide into two pars ① Regular monitoring survey and ② Case detection and treatment 2) Section of entomological survey for vector mosquitoes 3) Section of vector control 4) Section of social survey including education programs for villagers and concerned staff.

Besides, the regular meetings were held four times a year in which Japanese representatives including me, representatives from Ministry of Health, Jakarta, from Tropical Disease Center, Airlangga University, from Nusa Tenggara Barat (NTB) provincial health office and from two district health offices at West Lombok and Sumbawa participated. These activities evoked the following problems.

1) Malarea epidemiological conditions were different according to geographycal and environmental conditions. In Muninting area of West Lombok district two distinct malaria endemics were found, one in the coastal areas and the other in the forested and hilly areas.

2) Although we must adopt proper control methods based on endemic characters in each area, we have only limited number of control methods, and don’t have a fixed control manual for each character.

3) Malaria control activities are feasible only under systematic coopereation of various organizations, especially the comprehensive cooperation from villagers.

4) Intimate relationship between the central government and local governments must be established.

In the present project we are acting as a coordinator and as a financial supporter to establish a model of control strategy fitting for endemic conditions in each area. For this purpose we despatched two Japanese young staff for long stay after training them. This typr project will become important for our country to pactise medical cooperation or support in developing countries. However, the following problems in our country should be considered for further development of medical cooperation.

1) Shortage of specialists and practical personnel for control activities.

2) Low evaluation for persons concerning practical works in the field of preventive medicine including malaria control.

3) Shortage of educational organs to teach public health in developing countries.

S - V - 4) HASHIMOTO INITIATIVE AND OKINAWA INFECTIOUS DISEASE INITIATIVE:
JAPAN’S CHALLENGE TO GLOBAL PARASITE CONTROL

TSUTOMU TAKEUCHI

Department of Tropical Medicine and Parasitology, School of Medicine, Keio University

In May 1998, at G8 Summit in Birmingham, Mr. R. Hashimoto, the prime minister of Japan, made a proposal that the G8 should take a leading role in promoting parasite control efforts and contributing to the world health. Mr. Hashimoto’s proposal was endorsed and the resolution to this effect was incorporated into the official communique with the statement that “Control of parasitic diseases including malaria should be implemented”. Since then, Mr. Hashimoto’s proposal has been called “Hashimoto Initiative”(HI).

As the basic strategy, Hashimoto Initiative raised 4 issues. First, it stressed the necessity of effective international cooperation for parasite control. Than it raised the active pursuit of research as the scientific basis for parasite control and active implementation of the control projects. Finally, the initiative requested each of G8 countries to reinforce the capability to deal with parasitic diseases.

Among Japan’s own activities by HI, the most important contribution is the establishment of research and training centers for parasite control in Asia and Africa. As the
foothold for this purpose, Mahidol University in Thailand, and the Kenya Medical Research Institute (KEMRI) in Kenya, and the Noguchi Memorial Institute of Medical Research (NMIMR) in Ghana were chosen. These centers will have functions of effective training, technical support, operational research and information/human networking. To promote such activities, collaboration with international organizations will be accelerated. More importantly, the south-to-south cooperation will be strongly promoted. Among them, the center at Mahidol University became functional earlier. It is called the Asian Center of International Parasite Control (ACIPAC). Those at KEMRI and NMIMR are called East and South African Center of International Parasite Control (ESACIPAC) and West African Center of International Parasite Control (WACIPAC), respectively, and have already initiated their own activities.

In addition to HI, Japanese Government launched Okinawa Infectious Disease Initiative at the Kyushu-Okinawa G8 Summit in 2000, where HIV/AIDS, tuberculosis, malaria were listed as the major target of international collaboration. As the chairman country, Japan also proposed parasitic diseases and polio. In this sense, both Hashimoto Initiative and Okinawa Infectious Disease Initiative are expected to be effectively coordinated in near future, taking a leading role in parasite control at the global level.

S - V - 5) THE PROGRESS AND THE DIRECTION OF THE ASIAN CENTRE OF INTERNATIONAL PARASITE CONTROL PROJECT

NOBUHIKO NAGAI, NORIAKI TOMONO, MITSUHIKO IWASHITA and SOMEI KOJIMA

Asian Centre of International Parasite Control (ACIPAC)

At the G8 Summit Meetings in 1997 and 1998, former Prime Minister Ryutaro Hashimoto proposed that the G8 countries should cooperate to support countries affected by parasitic disease. Under the Hashimoto Initiative, Japan International Cooperation Agency (JICA) decided to set up one centre for parasitic disease control in Asia (Asian Centre of International Parasite Control, ACIPAC) and two in Africa. ACIPAC was launched as a five-year project in March 2000. The partner countries include the Greater Mekong Sub-region countries of Cambodia, Lao PDR, Myanmar, Thailand, and Vietnam. The overall goal is to strengthen parasite control programmes in the partner countries through human resource development. JICA’s regional technical cooperation programme supports ACIPAC activities.

The slogans “Save Wormy Schoolchildren” and “Save Schoolchildren from Parasites” have been chosen to promote this effort. ACIPAC will advocate parasite control through school health programmes. ACIPAC will utilize South-South Cooperation through information sharing and human resource networking, to help achieve the goal of parasite control.

In the second fiscal year (2001), the first three-month training programme “School-based Malaria and Soil-transmitted Helminthiasis Control for Programme Managers” was conducted from September 17 to December 7. Twenty-eight trainees who are administrators and technical officials from MoH, MoE and provincial Health Offices participated in this training. Five participants were invited from each of ACIPAC’s partner countries. There was one participant each from Japan, the UNICEF Laos office and the Kenya Medical Research Institute (one of the Hashimoto Initiative African Centres).

After the ACIPAC training, ACIPAC started a small-scale pilot project in each country. Planning for these pilot projects was done by the students during the ACIPAC International Training Course. In February and March 2002, as a first step, baseline surveys of malaria and parasite infection and socio-behavioral studies were conducted in Laos, Cambodia, and Vietnam. Myanmar will start its baseline study early next fiscal year. At the end of March, ACIPAC held an international workshop, and some of the graduates presented the results of baseline study.

In Thailand, ACIPAC has two model projects. They are in Ratchaburi province and Nakorn Si Thammarat province, which are endemic areas for malaria and soil-transmitted helminths, respectively. These model projects were utilized for field practice in the ACIPAC International Training Course.

ACIPAC is also planning an information-sharing network for parasitic disease control. ACIPAC has launched its Web site (http://www.tmacipac.mahidol.ac.th).
S - V - 6) ROLES AND CHALLENGES OF JAPAN’S ODA FOR GLOBAL HEALTH

OSAMU KUNII
Economic Cooperation Bureau, Ministry of Foreign Affairs, Japan


The Okinawa Infectious Diseases Initiative (IDI), reinforcing the Hashimoto Initiative, has six basic strategies: (1) Supporting institutional building of health sector with ownership of the recipient countries, (2) Focusing human resources development, (3) Strengthening partnership with civil society, donor countries and international organizations, (4) Supporting South-South cooperation, (5) Promoting research activities, (6) Promoting public health approaches at the community level.

[Achievements] IDI pledged US$ 3 billion in five years and has already spent US$ 1 billion in 1.5 year. It has provided over 50 bilateral projects and has contributed to international organizations such as WHO, UNICEF, UNAIDS, and to Japanese/International NGOs. In particular Japan established “Policy and Human Resource Development Fund (PHRD Fund)” in World Bank, “Human Security Fund” as the trust fund of UN to support their programs, which have been made use of over 100 countries. Besides IDI, Japan has pledged US$ 200 million to the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), 2nd largest contribution following the U.S.

[Challenges] More collaboration with stakeholders at the international/national/regional level is required to make most of Japan’s ODA. Especially it is crucial to make systematic and strategic coordination in (a) human resources from Japan Overseas Cooperation Volunteers (JOCV), NGOs to professionals, (b) organizations from JICA/JBIC to private enterprises, (c) programs from infrastructure buildings to community development. Japan could contribute to global partnerships to promote cooperation in development assistance.

I hope the Japanese Society of Tropical Medicine would play more active role in Japan’s ODA and NGOs’ assistance, providing technical assistance, researches directly contributing to ODA, and human resource development of the tropics and Japanese young generation.

S - VI - 1) DIFFICULTIES ENCOUNTERED IN FIELD RESEARCHES, WHICH WERE USED AS EXCUSES

MASAAKI SHIMADA
Research Center for Tropical Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

For the symposium I was given an assignment. What the chairperson requested me to talk was my personal experience in field works. Therefore the subjects I point out here are mostly on my personal experience.

1. Selection of target population
   As is usual in any field work, target population cannot always be selected based on scientific evidences. A community is usually selected according to the administrative boundaries and therefore a political will always influences the selection. Sample size may not be satisfactory. Sampling methods we apply sometimes do not work well because of the complaint of both selected and unselected people.

2. Members of the population
   After the selection, several persons or one in the community are recruited as informants or field workers. It is sometimes difficult to choose the best person by the research team. There is always a hierarchy in any community, which may disturb the best selection.

3. Identification of individuals
   It is not easy to identify a person at different times and places. Demographic data such as names, sexes, birthdays sometimes do not help because different names and birthdays could be used at different circumstances. Even pictures may not help especially in case of children. Population movement is another factor that makes the identification difficult.

4. Expectation of the community
What we are expecting in the field research and that the community is expecting in the work are sometimes different. Not all of the members of the community are cooperative. The demand of the community, excessive expectations and the existence of uncooperative people may influence the results of the study.

5. Heterogeneity of the population

The target population is not homogeneous like laboratory animals. This heterogeneity is a treasure of field work to obtain interesting results. However it is not easy to collect the data that properly show the heterogeneity of the population. There are usually limitations in collecting data on the characteristics of individuals.

S - VI - 2) FROM VOODOO EPIDEMIOLOGY TO RELIABLE EPIDEMIOLOGY

TAKESUMI YOSHIMURA

Dept. Clinical Epid., Inst. of Industrial Ecological Sciences, Univ. of Occupational and Environmental Health, Japan

An epidemiological study is essential and inevitable in the field study of tropical medicine, because an epidemiological study should only be the method to obtain necessary information for the people live in the target area. But an epidemiological study is not so appreciated in the society of tropical medicine because of uncertainty of date. In this presentation, four points will be discussed in order to establish reliable epidemiology.

1. Two types of epidemiological studies should be distinguished. One is descriptive type epidemiological study (to describe epidemiological characteristics of target population in a specific area.) The another is hypothesis testing type epidemiological study (to test hypothesis among human subjects). So called epidemiological study can be referred as one of two types. Study type should be clarified for further discussion.

2. Goal of the study should be clarified. For descriptive type epidemiological study, representativeness, degree of data accuracy should be carefully considered.

3. Study design should be carefully considered. Study design should be a balance between informativeness and feasibility of the study. Which level of information should we obtain for further action? Feasibility of a study (human subjects availability, cooperability, human resources, money, time, etc.) is fundamental for operation of the study.

4. Obtained results should be criticized for reasonable interpretation in terms of three points of view.

1) Is there any biases (Information bias, Selection bias)?
2) Are confounding factors in the study controlled properly?
3) Are statistical test and power problems properly considered?

For reliable epidemiology rather than Voodoo epidemiology, four points stated above should be considered.

S - VI - 3) FILARIASIS CONTROL: HOW WERE BLOOD COLLECTION AND MASS DRUG TREATMENT DONE?

EISAKU KIMURA

Dept. Parasitol., Aichi Medical Univ.

In 1978-81, I worked for the filariasis research and control program in Samoa as a WHO staff member. Blood collection by finger prick and venipuncture was repeated day after day. Under the WHO project, I never thought of ethical issues in relation to blood sampling, and cannot remember such things were discussed among WHO staff. As an international organization, there should never be any unethical affairs with WHO activities. It was an established
fact. In Samoa, there is a “matai” system, where decision by village matais, or chiefs, will overpower individual opinions of villagers. If matais consent blood collection, no villagers can protest. Although it may not be unethical, our program apparently took advantage of the system to carry out researches more easily than in other countries.

In 1973-75, I worked in Ethiopia for smallpox eradication program under WHO. What we did was to vaccinate as many children as possible. We worked hard in very remote mountainous areas where people often could not expect even the minimum level of medical care. In 1980, WHO proudly declared the eradication of smallpox, and published the final report. One sentence in it was shocking: Vaccination will no longer be justified because it has serious side reactions which may be fatal. We vaccinated many children without discussing any about the danger of the vaccine.

Was it ethically acceptable to vaccinate when we know some could suffer from serious reactions and they could never be attended? Can some people be sacrificed if the aim is for the international benefits and betterment of all people? We know that benefits are often more for the rich than the poor.

Ethical standards are moral prerequisite for all researchers, but for some civilized people, they have other aspects of avoiding legal complications, and self-satisfaction. It will be very important for us to participate positively in the formation of morally sound and practically workable standards with which field studies in developing countries can be performed with more confidence of scientific contribution.

S - VI - 4) BIOETHICS AND BIOMEDICAL RESEARCH IN DEVELOPING COUNTRIES: WHAT SHOULD RESEARCHERS DO FROM THE VIEWPOINT OF BIOETHICS?

Satoshi Kaneko
Cancer Information and Epidemiology Division, National Cancer Center Research Institute

The fundamental principle of ethics for medical research on human body had been based on protection of human right, and voluntarily participation to research. Along with rapid changes in modern societies, new concepts on human right have appeared on the field of biomedical research; that is, right to be let alone and right to control personal information. Furthermore, a process of informed consent to support voluntarily participation has been weighted in recent biomedical research. These new ethical issues have been considered to conflict with procedures in biomedical research. To balance the friction between them, several ethical guidelines for biomedical research using human subjects have been published based on the Declaration of Helsinki. Such guidelines recommended strengthening the review function of ethical review committees (ERCs) to make an applied biomedical research ethically justifiable. Although the ethical acceptability of biomedical research in developing countries supported or undertaken by developed countries must have followed the guidelines, it is not well discussed until the debates in 1997 on the zidovudine (AZT) clinical trials conducted in developing countries. These trials were implemented to see whether zidovudine (AZT) treatment for HIV-infected women prevented perinatal transmission of HIV. Although these debates resulted in the revision of the Declaration of Helsinki and an ethical standard to clinical trials in developing countries were given, they did not expand to ethical issues in general biomedical research or genomic research in developing countries.

In Japan, the bioethical guideline for epidemiological research has recently proclaimed by governmental ministries. Since the guideline has just come into effect, there has been no standard on ERCs and the abilities to review study protocols are not well evaluated. When it comes to ethical issues for research in developing countries, the situations becomes more chaotic. Most institutional ERC members do not know the situation in developing countries. Thus researchers must make an effort to inform the particular environments in developing countries to the ERC members. Sometimes, researchers might have to scientifically or logically assert reasons to ERC members when the protocol could not exactly follow the bioethical guidelines.

Through such constructive debates, the study protocol would become more refined, and persuasive. Mutual evolution of ERC members and researchers would not only promote the bioethical standard, but also improve the quality of the biomedical studies in developing countries. It is time for ethics and science to shake hands. The Japanese Society of Tropical Medicine must take a measure to enhance such mutual evolution.
W - I - 1) PREVENTIVE BEHAVIOR AND MORBIDITY OF JAPANESE TOURISTS FOR AFRICA

HITOSHI KIKUCHI, AKIHIRO HITANI, YASUHIRO ARAI, TOMOAKI SANO, TSUTOMU YOKOTA AND YOSHIE TANAKA

Narita Airport Quarantine Station

In many advanced countries, travel medicine is developed and travelers who visit risk areas can access travel medicine information. But, in Japan, travel medicine is developing and many tourists visit risk areas without or insufficient travel medicine knowledge. To assess the preventive behavior and health risks of Japanese tourists, a questionnaire study was carried out. Japanese tourists for Africa are focused. With the cooperation of a tour company, three hundred tourists of African tours were asked to complete questionnaire. 135 questionnaires were collected and were analyzed. Male was 58(37%) and the average age was 40.9 ± 12.4(mean ± SD), ranged from 8 to 76. As the infectious disease information sources, 96(62%) tourists get information from guidebooks, 66(42%) from tour agency, 36(23%) from internet web, and 30(19%) from friends. TRAVELER’S DIARRHEA: The attack rate of diarrhea was 24% and food score proposed by Sonneburg was 4.7 ± 2.6. Compared with the Sonneburg’s data of Kenyan travelers that the attack rate was 65.7% and the food score was 5.5 ± 8.6, the attack rate of Japanese tourists was lower. MALARIA. The knowledge of malaria was asked and 130(80%) knew malaria and 131(84%) intend to prevent for malaria. To prevent malaria, 117(87%) planned to use repellent before travel and 105(78%) actually used. 94(70%) planned to wear long sleeved and 86(63%) were, 93(69%) planned to use mosquito coil and 104(77%) used, 26(19%) planned to use mosquito net and 61(45%) acutally used. TRAVELER’S DIARRHEA: The attack rate of diarrhea was 24% and food score proposed by Sonneburg was 4.7 ± 2.6. Compared with the Sonneburg’s data of Kenyan travelers that the attack rate was 65.7% and the food score was 5.5 ± 8.6, the attack rate of Japanese tourists was lower. MALARIA. The knowledge of malaria was asked and 130(80%) knew malaria and 131(84%) intend to prevent for malaria. To prevent malaria, 117(87%) planned to use repellent before travel and 105(78%) actually used. 94(70%) planned to wear long sleeved and 86(63%) were, 93(69%) planned to use mosquito coil and 104(77%) used, 26(19%) planned to use mosquito net and 61(45%) acutally used. 28(20%) planned to take malaria prophylaxis before travel, 13(9.6%) actually took, 12(8.9%) take regularly, 5(3.7%) stopped after return. These indicate that most of the tourists know malaria and try to prevent mosquito bite, but the knowledge of malaria prophylaxis is insufficient. VACCINE: Vaccination rate was as follows: yellow fever 108(80%), tetanus 12(8.9%), rabies 6(4.4%), hepatitis A 5(3.7%).

These data support that guidebooks and the travel agency made a major role for tourists’ prevention. Tourists have tendency to keep general preventive measures. The travel agency recommended general precautions actively, but had difficulty to recommended medical prevention because they could not treat medical problems and travel medicine facility is very limited in Japan.

W - I - 2) KNOWLEDGE AND PREPARATION REGARDING THE RISK OF MALARIA AMONG JAPANESE INTERNATIONAL TRAVELERS

KAWAKAMI KEIKO1, KIMURA MIKIO2, HASHIMOTO MICHIKO3, AOKI KAZUKO4, HAMADA MASARU1, TANIHATA TAKEO1

1National Institute of Public Health, Tokyo, 2National Institute of Infectious Diseases, Tokyo, 3Tokyo Quarantine Station, Tokyo, 4Osaka Quarantine Station, Osaka, Japan

BACKGROUND: Although Japanese citizens travel increasingly to malaria-endemic areas, little has been described on their knowledge and preparation regarding the risk of malaria. This prompted us to conduct a questionnaire study on these issues targeting this population.

METHODS: During a 2-month period, travelers ≥ 16 years of age visiting Tokyo or Osaka Quarantine Station for vaccination were asked to answer a self-administered questionnaire. Most of them attended to receive yellow fever vaccine and therefore, scheduled to visit areas that are, to a greater or lesser extent, malarious. Questions included knowledge of the transmission route, main symptom and primary preventive measure of malaria. They were also asked whether and how they were informed of the current malaria situation at their destinations, what they would do in case of common cold symptoms after return, and whether they know a medical facility they should visit in case of suspected malaria.

RESULTS: Among a total of 468 individuals visiting the quarantine stations, 284 (61%) responded the questionnaire, with the response rate in Tokyo (questionnaires not delivered individually) and Osaka (delivered individually) being...
40% and 99%, respectively. Of those, 48% cases were subjected to analysis. Those who know the transmission route (mosquito bite), main symptom (fever) and primary preventive measure (avoiding mosquito bite) of malaria accounted for 85%, 83%, and 69%, respectively. Eighteen percent stated that the primary preventive measure is vaccination. Only as few as 40% knew whether their destination is malarious, 19% would visit a hospital specialized for infectious diseases in case of common cold symptoms, and 19% knew a medical facility they should visit in case of suspected malaria.

Very few travelers (11%) got the information from travel agencies on malaria prevalence in their destinations. **CONCLUSION**: Japanese international travelers visiting malarious areas have moderate knowledge of malaria; however, their preparation for the disease is unexpectedly insufficient and needs to be improved by education.

### W - I - 3) DISEASE TRENDS AMONG JAPANESE PEOPLE LIVING IN SOUTHEAST ASIA

AKIRA UCHIKOSHI, EIICHI OKUZAWA, NAOKO HONDA, NORIHKO OOKUBO, ATSUO HAMADA

Labor Welfare Corporation. Japan Overseas Health Administration Center, Japan Overseas Medical Fund

**Purpose and Method:** The number of Japanese people living abroad is increasing every year. We conducted research at two different hospitals in South East Asia (Ram Hospital in Chiang Mai, Thailand, and the Subang Jaya Medical Center in Kuala Lumpur, Malaysia) and have investigated disease trends among adult Japanese patients. We gathered disease names of adult Japanese (≥ 16 years old) who visited outpatient clinic in both hospitals from January 2000 to December 2001, and the data was classified according to the International Classifications of Diseases (ICD-10).

**Results:** At Ram Hospital the research covered a total of 4315 Japanese patients. The most common were respiratory diseases (739 cases, usually including respiratory tract infections), which was followed by digestive tract diseases (652 cases, often including dental caries) and infectious diseases (456 cases, many including traveler’s diarrhea). At Subang Jaya M. C., the research period included 4570 Japanese patients. The most common were respiratory diseases (239 cases, mostly respiratory tract infections), which was followed by ocular diseases (237 cases, showing symptoms of sore and/or swollen eyes) and unclassified clinical symptoms (237 cases, often including fever probably due to respiratory tract infections). During times of seasonal change, respiratory diseases were common in both hospitals during dry periods. At Ram Hospital, many patients suffered from infectious diseases during the monsoon season. At Subang Jaya M. C., patients suffered from ocular diseases during the dry seasons.

**Conclusion:** Most of adult Japanese visited the hospitals because of acute diseases such as respiratory tract infections, traveler’s diarrhea, dental caries and sore eyes. However, the number of Japanese who visited the hospitals for chronic diseases (i.e. metabolic disorders or circulatory diseases) was low. During the research period, we offered health consultations to adult Japanese living in the cities, and many of them suffered from the above-mentioned chronic diseases. It is necessary to urge them to visit the hospitals for taking medical cares of the diseases.
Despite a significant number of cases with tropical and parasitic diseases, major drugs for those diseases including several antimalarials are not licensed in our country. The foundation of our research groups that import, store and supply those orphan drugs dates back to the year 1980. The present research group, the Research Group on Chemotherapy of Tropical Diseases, is funded by the Japan Health Sciences Foundation. Recently, the number of institutions we designated to store and supply the orphan drugs has been increased to a total of 23 throughout our country, aiming that every Japanese physician who needs the drugs can have access without significant delay. We also initiated communications with some European hospitals that are specializing in tropical and parasitic diseases in order to update trends of the worldwide drug usage. We also publicized our web page on which the drugs we introduced, institutions storing those drugs and other useful information on travel-related illnesses are described. On our closed listserv our member physicians are discussing various issues, e.g. the availability of some drugs from other member physicians and the diagnostic problems of actual cases.

Until October 2001 when mefloquine was marketed, almost all mefloquine that was used for the treatment of falciparum malaria in our country must have been supplied from our research group, as chloroquine and primaquine are still now in the case of vivax malaria. Lives of many patients with severe malaria have been saved by injectable quinine, and recently with artesunate, supplied from our research group. In addition, we recently experienced four cases of severe amebic dysentery, three of which were associated with HIV infection, that showed dramatic improvement after receiving injectable metronidazole supplied from our research group. We also succeeded to introduce three kinds of drugs for African trypanosomiasis from a responsible section of WHO.

Thus, we are intending to grow our research group network into a comprehensive one in which we, not only supply the drugs upon request, but also accept and manage consultations regarding various clinical issues of tropical and parasitic diseases including diagnostic and therapeutic matters.
accomplished, and then transferred to the malaria zone, where is convenient to cultivation. We detected the 3.7 kb deletion type of α-thalassemia (-α3.7) with a high gene frequency, 0.54 in the Danuwars, while only 0.05 in the Tamangs, 0.14 in the Parbates and 0.28 in the Newars, Haplotype analysis of α-globin gene cluster showed that the α-thalassemia mutation has four different genetic background in the Danuwars. These results suggest that the high frequency of α-thalassemia in the Danuwar is due to the adaption to malarial environment rather than to events such as a bottleneck.

To examine the relationship between α-thalassemia and the actual malaria infection, we analyzed the malaria infection rate by genotype in the malaria zone mentioned above since 2000. In September 2000, 3 of 12 (25.0%) in αα/αα, 1 of 35 (2.9%) in -α3.7/αα and 5 of 37 (13.5%) in -α3.7/-α3.7 were positive for plasmodium by PCR. In March 2001, 20 of 63 (31.7%) in αα/αα, 5 of 38 (13.2%) in -α3.7/αα and 5 of 26 (19.2%) in -α3.7/-α3.7 were positive, and in February 2002, 21 of 82 (25.6%) in αα/αα, 6 of 39 (15.4%) in -α3.7/αα and 2 of 29 (6.9%) in -α3.7/-α3.7 were positive for plasmodium. These results showed that the malaria infection rate was significantly lower in the population with α-thalassemia than those without α-thalassemia.

W - II - 2) KNOWLEDGE AND PREPARATION REGARDING THE RISK OF MALARIA AMONG JAPANESE INTERNATIONAL TRAVELERS

KAWAKAMI, K.

W - II - 3) MOLECULAR ANALYSIS OF CHLOROQUINE RESISTANT RELATED GENE IN MALAWI

TOSHIHIRO MITA1, AKIRA KANEKO1, LUN J KOJIRO1, BWIJO BWIJO1, TAKEKI MIHO1, INNCENT L ZUNGU2, TSUKAHARA TAKAHIRO1, and KOBAYAKAWA TAKATOSHI1

Department of International Affairs and Tropica Medicine, Tokyo Women’s Medical University School of Medicine1, Community Health Sciences Unit, Ministry of Health and Population2

Although chloroquine resistant Plasmodium falciparum has been spread in Africa since 1970, chloroquine is still important compound because of its inexpensiveness and safeness. In Malawi, first line drug for treatment of P. falciparum was officially replaced from chloroquine to sulfadoxine/pyrimethamine in 1993 because of high rate of treatment failure (80%) and in vitro resistance (48%). To date, the use of chloroquine has been strictly enforced. We conducted two drug efficacy surveys of in vitro in 1998 and in vivo in 2000 at Salima district in Malawi and found significant reduction of chloroquine resistant isolates both in vitro (3%) and in vivo (9%).

We assayed two genetic mutations implicated in chloroquine resistance (pfmdr1 and pfcrf) from 128 P. falciparum isolates collected during the 1998 and 2000 resistance studies P. falciparum DNA was extracted from filter paper with finger prick blood Nested PCR followed by RFLP methods were performed to determine pfcrf polymorphisms at position 76, 220, 271, 326, 356 and 371, and for pfmdr1 at position 86, 184, 1034, 1042 and 1246.

In pfcrf, mutant type alleles were detected in 2 to 21% of isolates at position 76, 220, 271, and 371. The prevalence of mutant alleles significantly decreased from 1998 to 2000 at position 76 (P=0.007), 220 (P=0.009), 271 (P=0.007) and 371 (P=0.007). Nearly all of mutant alleles were detected in isolates with mixed infections; i.e. including both wild and mutant. In pfmdr1, high prevalences of mutant type alleles were detected at position 86 and 184. No difference was found in these prevalences between 1998 and 2000. In pfcrf, Fst value were significantly high and similar level (0.051-0.055) at position 76, 220, 271, and 371 between two populations (P<0.02). Contrary to pfcrf, the Fst value at position 86, 184, 1042, and 1246 were not significant in pfmdr1. These results suggested that pfcrf has four distinct regions (76, 220, 271, and 371) of loci with respect to allele frequency between parasite population in 1998 and 2000.

Our results support one possibility that the elimination of chloroquine pressure has reduced the prevalence of mutant pfcrf alleles and resulted in a substantial recovery of chloroquine sensitivity. Furthermore, in addition to the K76T polymorphism chloroquine sensitivity is highly dependent
on other as yet unidentified polymorphisms or loci.

W - II - 4) GENERATION OF TRANSGENIC ANOPHELINE MOSQUITOES EXPRESSING A GENE ENCODING ANTI-MALARIA MOLECULE

SHIGETO YOSHIDA1, TONY NOLAN2, FLAMINIA CATTERUCCIA3, HIROYUKI MATSUOKA1, CHISATO SEKI1, DAI SUKE KONDOH1, AKIRA ISHI1

1Departments of Medical Zoology, Jichi Medical School, 2Department of Biological Science, Imperial College of Science, Technology and Medicine

The recent generation of genetically transformed mosquitoes has raised hopes for the production of mosquito strains that are unable to transmit various parasites and viruses.

We have previously constructed a gene encoding of a single-chain antibody fragment (scFv) specific for Pbs21 molecule expressed on the ookinete surface. The scFv, designated 13.1 scFv, bound to the surface of *P. berghei* ookinete, and blocked oocyst development in the mosquito midgut by at least 93%, as assessed by oocyst counts in mosquitoes. To examine whether expression of 13.1 scFv in the mosquito midguts could lead to transmission-blockade of malaria in the mosquito stage, we generated 5 lines of transgenic *Anopheles stephensi* mosquitoes that express the 13.1 scFv-Shiva gene under the control of the gut-specific and blood-inducible *An. gambiae* carboxypeptidase (AgCP) promoter using *Minos* germ line transformation. The embryos were microinjected by using glass needles with a mixture of *Minos*-13.1 scFv-Shiva transfer plasmid and *Minos* transposase plasmid. The *Minos* transposase mediates integration into the genome of *An. stephensi* cells. Hatched larvae were analysed on an inverted microscope at a wavelength of 490 nm to detect EGFP expression.

Real-time PCR analysis showed that expression of 13.1 scFv-Shiva mRNA is specifically induced (8-fold) in the guts of transgenic mosquitoes, with peak expression at ~3h after a blood meal. By 48h after a blood meal, mRNA abundance returns to a level close to that present before a blood meal. By 48h after a blood meal, mRNA abundance returns to a level close to that present before a blood meal. To examine inhibition of oocyst formation in the transgenic lines, wild-type and transgenic mosquitoes were allowed to feed on the same *P. berghei*-infected mouse. Oocyst formation in one of transgenic lines were significantly reduced (50%) compared with that in wild-type.

W - III - 1) INTRANASAL INOCULATION OF YEAST-PRODUCED MALARIA OOKINETE SURFACE PROTEINS CO-ADMINISTERED WITH CHOLERA TOXIN INDUCES COMPLETE TRANSMISSION-BLOCKING IMMUNITY AGAINST A RODENT AND A HUMAN MALARIA PARASITE

AYANO KISHIMOTO1, TAKAFUMI TSUBOF, MAYUMI TACHIBANA2, TAKESHI ARAKAWA1, JETSUMON P. SATTABONGKOT4, NANTAVEDEE SUWANABUN4, THANAPOM RUNGRUANG2, HAJIME HISAE D3, ANTHONY STOWERS5, YASUNOBU MATSUMOTO2, NAOTOSHI TSUJI7, YOSHIYA SATO6,9, and MOTOMI TORII2


Transmission-blocking Vaccines against sexual-stage malaria parasites have been considered one strategy for malaria control. Recently, several genes encoding ookinete surface proteins (OSP) have been cloned from *Plasmodium* species of rodent, human and avian malaria parasites. To investigate whether mucosal vaccines against malaria para-
site OSPs are feasible strategy for induction of systemic transmission-blocking immunity, the yeast-synthesized \textit{P. yoelii} Pys25 protein was administered to DBA/2 mice through oral or nasal route in combination with cholera toxin (CT). Intranasal administrations of Pys25 in the presence of CT induced strong systemic humoral immune responses evidenced by high levels of serum IgG1 with low but detectable levels of IgG2a antibodies, suggesting that Th-2 type immune responses were predominantly induced against Pys25 protein. However, in the absence of CT the intranasal immunization failed to induce any detectable levels of serum antibodies. Oral administrations of Pys25 even in the presence of CT did not induce antibody responses. When \textit{Anopheles stephensi} mosquitoes were allowed to feed on mice infected with \textit{Plasmodium yoelii} 17X, which previously vaccinated intranasally with Pys25/CT, oocyst formation in mosquito midgut was completely blocked in every engorged mosquitoes examined. Further, passive transfer of immunesera to naïve mice conferred complete transmission-blocking immunity. Similarly, intranasal immunization of mice with \textit{Plasmodium vivax} ookinete surface protein, Pys25 induced significant serum IgG with high IgG1/IgG2a ratio. Feeding \textit{Anopheles dirus} mosquitoes with mixtures of immunesera and gametocyteic blood derived from vivax volunteer patients in Thailand significantly reduced both the number of midgut oocyst as well as the percentage of infected mosquitoes. The observed transmission-blocking effect was dependent on immunesera dilution. These studies demonstrate for the first time that the mucosally induced mouse immunesera against malaria ookinete surface proteins can completely block parasite transmission to vector mosquitoes, suggesting the possibility of developing non-invasive mucosal vaccines against malaria.

**W - III - 2)** HIGH EXPRESSION OF CHOLERA TOXIN B SUBUNIT PENTAMER IN METHANOPHILIC YEAST \textit{PICHIA PASTORIS} AND ITS ORAL IMMUNOGENICITY IN MICE

TAKESHI ARAKAWA¹, JIN YAH¹, AYANO KISHIMOTO², YASUNOBU MATSUMOTO³, NAOTOSHI TSUJI⁴, HHIROMU TOMA⁵, and YOSHIYA SANO⁵

¹Division of Molecular Microbiology, Center of Molecular Biosciences, University of the Ryukyus,
²Department of Bio-production, School of Agriculture, University of the Ryukyus,
³Laboratory of Global Animal Resource Science, Graduate School of Agricultural Life Sciences, University of Tokyo,
⁴Laboratory of parasitic diseases, National Institute of Animal Health, National Agricultural Research Organization,
⁵Department of Parasitology, School of Medicine, University of the Ryukyus

Cholera toxin B subunit (CTB) has been expressed in bacterial gene expression systems and successfully used for the induction of mucosal and systemic immune responses to chemically or genetically conjugated antigens. In the present study we tested the possibility for yeast to express CTB gene and evaluated the feasibility of recombinant yeast-based oral immunization strategy. We introduced into \textit{Pichia pastoris} chromosome 1) the authentic CTB gene (CTB\textsuperscript{a}), 2) CTB gene deleted with 22-amino acid leader peptide coding sequence (CTB\textsuperscript{d}), and 3) CTB gene fused at the C’ terminus with yeast microsomal retention signal (HDEL)-encoding sequence (CTB\textsuperscript{HDEL}). We found significant levels of CTB\textsuperscript{a} and CTB\textsuperscript{HDEL} pentamers retaining \(G_{4.4}\)-ganglioside affinity were produced and secreted into the medium (30 \(\mu\)g/ml). We detected CTB\textsuperscript{d} transcript by RT-PCR but the protein was not detected. Yeast-synthesized recombinant CTB was found heat-labile, quickly dissociated into monomers when cells were heat-treated at 100 °C, but the protein was almost completely resistant to desiccation. Next, to evaluate oral immunogenicity of yeast-produced CTB, yeast or culture supernatant calculated to contain 8-16 \(\mu\)g of pentameric CTB was given to mice by oral gavage once a week for 6 weeks. Mice fed yeast cells, but not with culture supernatant, developed CTB-specific serum IgG (predominantly IgG1) and intestinal S-IgA antibodies. Feeding 10 \(\mu\)g of commercially available CTB protein mixed with untransformed yeast cells induced lower level of CTB-specific serum IgG, indicating that recombinant CTB accumulated within yeast cells induced lower level of CTB-specific serum IgG, indicating that recombinant CTB accumulated within yeast cells has higher oral immunogenicity than naked CTB, probably due to increased resistance to digestive degradation in gastrointestinal tract. The induced antibody response was maintained at least for six months when mice were given extra booster feedings of yeast cells. Taken together, these results suggest that yeast can be used as an efficient vaccine production system as well as an oral delivery system for vaccination.
W - III - 3) EXPRESSIONS OF CYTOKINES AND MONOSACCHARIDE TRANSPORTERS IN THE DUODENAL MUCOSA OF GASTRO-INTESTINAL SYMPTOMATIC PATIENTS IN THE RURAL AREA OF THAILAND

SUJET LERTANEKAWATTANA 1, THAVORN WICHATRONG 2, KAMRON CHAISAIRI 3, RYUICHI UCHIKAWA 4, AND NAOKI ARIZONO 5

Nongkhai Hospital, Thailand 1, Nongkhai Provincial Health Office, Thailand 2, Department of Medical Zoology, Kyoto Prefectural University of Medicine, Japan 3

Expression levels of cytokines and GLUT family monosaccharide transporters in the duodenal mucosa were examined in patients from Nongkhai, Thailand, who had received gastrofiberscopy because of the gastrointestinal problems. Duodenal biopsy specimens were collected from a total of 33 patients (24 males and 9 females, 45.0 ± 13.5 years old): 10 patients showed present or recent intestinal helminth infections, including strongyloidiasis, taeniasis and ascariasis (group A), 7 were urea-test positive, indicating Helicobacter pylori infection (group B), and 16 had neither helminth infection nor urea test positivity (group C). Total RNA was extracted from the biopsied specimens and semi-quantitative RT-PCR was performed. Positivities of IL-13, IL-5 and IFN-γ mRNA expressions in all patients were 24.2, 60.6 and 100%, respectively, with the highest IL-13 and IL-5 positivities in group A followed by group C and B. IL-5 positive rate was also significantly higher among patients with high peripheral blood eosinophil counts (>4%) than in patients with low peripheral blood eosinophil counts. GLUT-1 and GLUT-5 were detectable in all patients. Although GLUT-1 expressions did not differ between group A, B and C, GLUT-5 expressions were significantly lower in group B than in group C. These results indicate that helminth and H.pylori infections result in different immunopathological responses in the duodenal mucosa: lower expressions of type 2 cytokines and monosaccharide transporters in H.pylori infection than in helminth infections.

W - III - 4) STUDY OF ASCARIS AND ULCERATIVE COLITIS-GENETIC ANALYSIS OF CROSS-REACTIVE ANTIGENICITY BETWEEN ASCARIS LUMBRICOIDES HOMEINIS ANTIGEN AND HUMAN COLON MUCOSA

HAYASHI ITOKO, KANDA SEJI, YOSHIDOME ATSUO, ISHIDA TAKAAKI, NISHIYAMA TOSHIMASA

Dept. of Public Health, Kansai Med. University

It is known that the antibody against the Ascaris is produced in patient’s serum of ulcerative colitis (UC). We have already reported the cross-reactive antigens between A. lumbricoides and human colon mucosa are presented. In this study, to determine the genes related with cross-reactive antigens between A. lumbricoides and human colon mucosa, we analysed the screening method at the DNA levels using the human colon cDNA library (Clontech laboratories Inc., CA,USA). λ phage was infected to E. coli XL-1 Blue, and IPTG induced-protein derived from each plaques were transferred to the membrane and they were investigated by immuno-staining method using the anti-A. lumbricoides rabbit serum. We analyzed 50,000 plaques approximately, 3 clones were reacted positively as a result of secondary screening. To determine the sequences of these clones, each cDNA was analyzed by the DNA sequencer ABI PRISM 310 Genetic analyzer. As the result, one was considered to be a transmembrane mucin 12 homolog. Other two genes were similar to actin, gamma 2 of enteric smooth muscle and beta-casein-like-protein. Mucin exists in the digestive organs, especially in the colon and was reported that the antibody against colonic mucin might contribute to the epithelial cell injury of colonic mucosa through antibody-dependent cell-mediated cytotoxicity (ADCC) and this autoantibody levels are high in some patients for UC. Mucin12 gene contains in the coding regions that are homologous to mucin3, mucin4, mucin11 and mucin17 with high frequency. In these results, A. lumbricoides was likely to harbor in the human intestine, because mucin3 exists richly in the intestine, and it was guessed to be a suitable environment for A. lumbricoides.
W - IV - 1) FUSION DNA VACCINE AGAINST INFECTION WITH HIGH VIRULENT TOXOPLASMA

KAZUNARI ISHII¹, YOKO NAKANO², TOHRU SAKAI¹, SHINJIRO HAMANO¹, AKIHIKO YANO³, KUNISUKE HIMENO¹

¹Department of Parasitology, Faculty of Medical Sciences, Kyusyu University, ²Department of immunology & Parasitology, Tokushima University School of Medicine, ³Department of Infection Mechanisms and Immunity, The Graduate Course for Medical Doctor for Science, Chiba University School of Medicine

For most obligate intracellular protozoa such as Toxoplasma gondii (T. gondii) and Trypanosoma cruzi, available vaccines have not yet been developed, despite that those kinds of pathogen are resistant or less effective for chemotherapy in general. Cellular immunity especially CD8⁺ T cell response plays essential roles for the protection against those kinds of pathogens. Effective protective immunity against those infections is, however, hardly inducible by conventional vaccination using proteins/peptides derived from target pathogens. It is a well-established consensus that protein antigens must be ubiquinated and then processed by proteasome within antigen-presenting cells when MHC class I-restricted CD8⁺ T cell response is preferentially induced. From these bases, we fused naked DNA of SAG-1 (major surface antigen of Toxoplasma gondii) with that encoding ubiquitin. Thereafter, BALB/c mice were subcutaneously injected with this fusion DNA using gene gun before the infection with a high virulent RH strain of T. gondii. Strikingly, most mice vaccinated with the fusion DNA acquired strong protective immunity and controlled the infection, although DNA vaccine with either DNA encoding SAG-1 gene alone or that encoding ubiquitin gene alone was not effective. Furthermore, the effect of ubiquitination was confirmed in vitro using a specific inhibitor for proteasome, MG-132. Mice immunized with the fusion DNA showed a potent cytotoxic activity of CD8⁺ T cells specific for SAG-1 transfected target cells. This kind of vaccine strategy is expected to be useful for infections with other type intracellular pathogens including viruses and bacteria.

W - IV - 2) AN INTERLEUKIN-18 EXPRESSION PLASMID ADMINISTRATION INITIATES A PROTECTIVE T HELPER TYPE 1 IMMUNE RESPONSE AND RESOLVES CUTANEOUS LEISHMANIASIS

YONG LI¹, YOICHI MAEKAWA², SHINJIRO HAMANO¹, TOHRU SAKAI², KAZUNARI ISHII¹, MANXIN ZHANG² and KUNISUKE HIMENO¹

1.Department of Parasitology, Graduate School of Medical Sciences, Kyusyu University
2.Department Parasitology and Immunology, Tokushima University, School of Medicine

We investigated the therapeutic effect of an IL-18 expression plasmid administration on susceptible BALB/c mice infected with Leishmania major. BALB/c mice were subcutaneously challenged with L. major into hind footpad. Simultaneously, they were weekly treated with 4 µg IL-18 and/or IL-12 expression plasmid DNA with the gene gun. All of the treated mice showed similarly decreased footpad swelling and less parasite burden compared with those treated with empty vector (control mice). IFN-γ was significantly increased at 3 weeks after infection in all treated mice but not in control mice. Antibody administration demonstrated that CD4⁺ T cells and IFN-γ played critical roles in the protection against L. major induced by IL-18 expression plasmid administration. Moreover, TNF-α was also increased in the treated mice. In TNF-α-deficient BALB/c mice, which were also susceptible and rapidly succumbed to progressive disease after L. major infection, IL-18 expression plasmid administration did not lead to the resistance as in the wild type mice, despite of the high level of L. major-specific IFN-γ production. This demonstrated the critical role for TNF-α in L. major infection. These results suggest that administration of an IL-18 expression plasmid with gene gun is able to protect susceptible mice against an intracellular protozoan infection, with shifting of immune response towards Th1 direction.
3) PROTECTIVE ROLE OF IgE IN MICE INFECTED WITH PLASMODIUM BERGHEDI ANKA

TAKAHISA FURUTA¹, TAKANE KIKUCHI¹, MIKIO KIMURA² and NAOHIRO WATANABE³

Division of infectious Genetics, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo¹, Infectious Disease Surveillance Center, National Institute of Infectious Disease², Department of Tropical Medicine, Jikei University School of Medicine³

Recently, it has been reported that IgE is related to the pathogenesis in severe malaria infection, particularly in cerebral malaria. IgE levels in the sera of individuals living in high P. falciparum endemic areas were investigated, approximately 85% of people from these areas had significantly elevated level of total IgE. However, the role and biological significance of IgE in the protection against P. falciparum and pathogenesis of malaria are not known. For these reasons, we decided to examine the possible roles of IgE responses using IL-5 transgenic and SJA/9 mice, which are genetically producing high levels of IgE under normal condition or deficient of IgE production. 10^3 P. berghei ANKA-infected erythrocytes were injected i.v. into IL-5 transgenic and the background mice, and the parasitemia was measured every day. After the infection of P. berghei, although the parasitemia was increased gradually in both groups of mice, the parasitemia in IL-5 transgenic mice was always lower than that in background mice, and the IL-5 transgenic mice showed longer survival. We examined the protective role of IgE in P. berghei infection using congenitally selective IgE-deficient SJA/9 and the background SJL/J mice. After P. berghei infection, the parasitemia in SJA/9 mice was significantly higher than that of SJL/J mice, and the mortality of SJA/9 mice was also higher than that of SJL/J mice. These results indicated the protective roles of IgE for P. berghei infection. The production of anti-P. berghei specific IgE antibody in the P. berghei ANKA-infected mice was examined by Western blot analysis, the results showed that anti-P. berghei IgE antibody was also produced in the sera from these mice. Since IgE has both protective and pathogenic effects in infectious diseases via two major receptors, the high-affinity receptor FcεRI and low-affinity FcεRII or CD23, we examined the possibility that those two kind of receptors involved with IgE-mediated resistance against P. berghei infection using WBB6F1-W/W³ mice, which are congenitally deficient mast cells bearing FcεRI receptor and CD23 Ko mice. After P. berghei infection in W/W³ and CD23 Ko mice, the parasitemia in W/W³ and CD23 was always higher than those in the background mice, suggesting that the protective effect of IgE on P. berghei infection was mediated via FcεRI and RII.

4) THE ROLE OF ERYTHROCYTES ON SUPPRESSION OF IMMUNE RESPONSE IN MURINE MALARIA

MICHIKO TOKUNAGA, MINAKO OHNO, MAKOTO OWASHI

Department of Life Science, Faculty of Integrated Arts and Sciences, Tokushima University

It is well known that malaria is associated with hepatosplenomegaly, anemia and myeloid hyperplasia. The increased levels of hemopoiesis and the phagocyte system are thought to be implicated in this pathogenesis. Immune depression during malaria is also a well-recognized phenomenon that likely contributes on the host-parasite relationship. Previous studies have shown that suppressor T cells or macrophages are involved this phenomenon. Recently we found that RBC from Plasmodium-infected mice showed increased levels of affinity to some FITC-labelled plant lectins.

In the present study, we examined the possible role of RBC of Plasmodium-infected mice on the regulation immune response upon Plasmodium infection. Proliferative response of splenocytes upon stimulation with Con A or allogenic APC increased in the presence of RBC from normal mice, whereas depressed in the presence of RBC from P. yoelii-infected mice. Supernatant of RBC lysate showed similar effect on the proliferative response with the RBC. On the other hand, RBC lysate from P. yoelii-infected mice induced superoxide production from neutrophils. Time course study revealed that the stimulatory activity of the
RBC lysate coincided with the increase of parasitemia during *P. yoelii* 17X infection. The stimulatory activity of the RBC lysate was partially lost after the treatment with heparin-agarose. HPLC analysis equipped with G3000SW column indicate that the stimulatory factor possesses a molecular weight mass of around 14kd. The relationship between the depression of the immune response by RBC of *P. yoelii*-infected mice and superoxide production from neutrophils was discussed.

W - V - 1) APPLICATION OF QUESTIONNAIRES FOR MONITORING SCHISTOSOMA MANSONI INFECTION AFTER MASS-TREATMENT

TOMOKO KISU¹, YUJI AYAKA¹, EIKO KANEDA², KAZUHIKO MOJI³ AND MASAAKI SHIMADA¹

Research Center for Tropical Infectious Diseases¹, Department of Social Environment², Institute of Tropical Medicine, Nagasaki University

The purpose of the study is to determine if adding some questions about water-contact behavior on signs and symptoms improve the efficacy of questionnaires for the monitoring of *Schistosoma mansoni* infection.

<Subjects and methods>

A total number of 1033 schoolchildren aged 7-17 years in four primary schools in Moshi Tanzania were involved in the study. February 2001, they received mass treatment with praziquantel and mebendazol for the de-worming of schistosome and intestinal parasites. Before the treatment, the children were requested to answer a questionnaire. They were examined for the ova of schistosome and intestinal parasites in stool before the treatment and one month later. A year later in February 2002, the examination and questionnaire were carried out again. Only 267 children completed the examination and questionnaire. The questionnaire consists of the questions on the subjective symptoms in the previous two weeks and frequency and nature of their water-contact behaviors. The answers of questions recorded as 'yes', 'no' or 'I don’t know'. Stool examination was conducted with Kato-Katz procedure. The association between infection and each questionnaire variable was calculated by using a chi-square test and a logistic binomial regression analysis.

<Results>

1) The prevalence of *S. mansoni* infection was 67.4% before the treatment and decreased to 6.4% after treatment. In 2002, one year after the treatment, the prevalence increased to 27.0%. The mean intensities were 29.8 eggs/gram, 0.3 eggs/gram and 2.5 eggs/gram respectively.

2) Before treatment, constipation (OR=2.91, 95% C.I.; 1.29-6.58, P=0.010) and net fishing (OR=2.30, 95% C. I.; 1.29-4.11, P=0.005) were significantly associated with infection. Sensitivities and specificities were 20.6%, 89.7% and 43.5%, 70.6% respectively. One year after treatment, only diarrhea (OR=1.91, 95% C.I.; 1.10-3.32, P=0.021) had significant association with infection. The sensitivity and specificity were 61.1%, 54.9% respectively.

3) No water contact behavior had significant association with infection in this study.

4) When the factor of the previous infection before treatment was introduced to a logistic binomial regression analysis, both previous infection (OR=1.96, 95% C.I.; 1.04-3.70, P=0.037) and diarrhea (OR=1.88, 95% C.I.; 1.08-3.27, P=0.026) were significantly associated with the current infection.

In the study area, questions about water-contact behavior added on signs and symptoms did not improve the efficacy of questionnaires for the monitoring of *Schistosoma mansoni* infection.
RAPID SCHOOL HEALTH QUESTIONNAIRE SURVEYS ON URINARY SCHISTOSOMIASIS IN COASTAL KENYA AND ZANZIBAR

KAZUHIKO MOJI1, MOHAMED KARAMA2, NG'ETHE MUHOHO2, HABAB CHWAYA3,4, ALI-FOUM1, SHIMBA KHAMIS1, YUJI ATAKA1, MASAAKI SHIMADA1, AND YOSHIKA OKI1

Institute of Tropical Medicine, Nagasaki University1, Kenya Medical Research Institute2, Ministry of Health and Child Welfare, Zanzibar3, now UNICEF Zanzibar4

The WHO’s resolution on schistosomiasis has set the goal of attaining a minimum target of regular administration of chemotherapy to at least 75% of all school-age children at risk of morbidity by 2010. However, systematic data on the prevalence of infection, the coverage of administration of chemotherapy and school health education have not been collected in Sub-Saharan African countries. Therefore, we conducted rapid school health questionnaire surveys in Kwale District of Coast Province of Kenya (K), and in the two islands of Zanzibar; Unguja (U) and Pemba (P) in 2001. In total, 264 schools (113 in K, 84 in U, and 67 in P) were participated in the study. Ten boys and ten girls in class 4 were asked to answer questions in these surveys. In Zanzibar trained health staffs visited schools and conducted the survey. In Kenya the procedure of survey was explained to teachers in a district meeting, and teachers themselves conducted the survey in their schools. The results were collected by mail. Questions asked were knowledge on schistosomiasis, presence or absence of self-diagnosed urinary schistosomiasis, subjective symptoms such as blood in urine, contact with river water in the previous two weeks, and experiences of urine examination, treatment, and health education. Mean percentages of children answering “yes” to questions in K, P, and U were as follows (sex combined): Knowledge on what schistosomiasis is, 73%, 50%, 31%; Self-diagnosed urinary schistosomiasis, 39%, 21%, 6%; Blood in urine, 33%, 19%, 6%; Contact with river water in the previous two weeks, 82%, 57%, 16%; Health education, 34%, 24%, 31%; Urine examination, 26%, 19%, 8%; Treatment, 26%, 35%, 11%. These results indicate the needs of systematic efforts to achieve the WHO’s goal. The governments must annually conduct this kind of rapid monitoring survey on health status of school children, coverage of school health services, and coverage of health education to accumulate the basic information on school health.

WATER CONTACT BEHAVIORS AMONG SCHOOLCHILDREN IN AN ENDEMIC AREA OF SCHISTOSOMIASIS, TANZANIA: A COMPARATIVE STUDY OF DIRECT OBSERVATION AND 24-HOUR RECALL METHOD

YUJI ATAKA1, EIKO KANEDA2, MASAAKI SHIMADA1, TOMOKO KISU1, KAZUHIKO MOJI1

Research Center for Tropical Infectious Diseases, Institute of Tropical Medicine, Nagasaki University1, Department of Social Environment, Institute of Tropical Medicine, Nagasaki University2

Estimation of exposure to cercarial infested water is critical for epidemiological and immunological studies in a schistosomiasis endemic area. Questionnaire surveys have been used to estimate water exposures, addressing frequency of water contact only, due to its cost-effectiveness and less labor-intensiveness. Direct observation needs more cost and time, but it provides more accurate exposure information. We compared the results of 24-hour recall method with direct observation method for clarifying whether 104 schoolchildren living in Lower Moshi area, a Schistosoma mansoni endemic area, of Tanzania could accurately recall their water exposures. Individual tracing method was applied to obtain detailed direct observation data for all water contact behaviors, and, on the following day, an interview was carried out to collect corresponding information on all contact behaviors. Exposure index (EI: %/min/day) was calculated by contact duration and intensity of exposure (percent body surface area) using Lund and Browder burn chart. The most critical water contact behavior for the infection was swimming/playing in the river and canal among schoolchildren. They used safe water, in most cases, from the tap or deep well for bathing purpose. We found a significantly smaller frequency (4.5 vs. 6.4, p<0.001), but longer duration (29.7 vs. 24.6 min, p<0.01), of water contact in the re-
call data than directly observed data. For the risk water contacts, the EIs of water contacts obtained by recall method were significantly higher than those of direct observation ($p<0.001$). The EIs of 24-hour recall were significantly correlated with those of direct observation ($\rho = 0.885$, $p < 0.01$); more strongly among boys ($\rho = 0.931$, $p < 0.01$) than girls ($\rho = 0.750$, $p < 0.01$). Although the values of the EI are significantly higher in the recall data and should be corrected in practice, these results show a possibility of replacing direct observation data by detailed recall data. The 24-hour recall method is a practical procedure to accurately quantify water exposure in Lower Moshi area where *S. mansoni* has been endemic.

**W - V - 4) SCHOOL-BASED AND RESIDENCE-BASED ANALYSES ON INFECTIONS WITH *S. HAEMATOBIUM* AND *S. MANSONI* AMONG CHILDREN IN MOSHI, TANZANIA**

EIKO KANEDA¹, TOMOKO KISU², YUI ATAKA², MASAAKI SHIMADA², AND KAZUHIKO MOJI²


Schistosomiasis in Africa is not widely and evenly spread along river lines, but is scattered in clusters in some particular areas. The areas vary in seasons and years, which seems to have influence upon the infection with the disease to human bodies. This paper is to report the characteristics of distributions for two kinds of schistosomes, *S. haematobium* and *S. mansoni*, both existing in the same area, one from the view of school districts and another from that of dwelling places.

Subjects for our survey were the children in grades four and five from Chekereni primary school in Chekerini, and those from Oria primary school in Oria school district, the number of which was 284 in total, the former 199, and the latter 85. Whether inflection was positive or negative was determined by examining existence of the eggs in subjects’ feces and urine. Kato-Katz method for *S. mansoni*, and nucleopore filter paper method for *S. haematobium* diseases were adopted.

Presence of *S. mansoni* eggs was positive among 50.8% of children from Chekereni primary school whereas 31.2% in Oria primary school. *S. haematobium* eggs were observed among 20% of children in Oria primary school, while only few children from Chekereni primary school were infected. No significant difference was observed on the prevalence of *S. mansoni* between Chekereni children coming from Chekereni area and those coming to the same school from Oria area ($P>0.05$). While for that of *S. haematobium*, a significant difference was observed ($P<0.05$).

Epidemiological surveys related to the infection with the parasites have often been carried out on the basis of school districts. The distribution of infection is often biased by endemic area. We conclude that location of subjects’ inhabitation should also be considered for epidemiological surveys on parasitic diseases.

**W - VI - 1) CHARACTERIZATION OF THE cDNA OF THE MIRACIDIAL ANTIGEN FAMILY OF *SCHISTOSOMA JAPONICUM* (CHINESE MAINLAND STRAIN).**
W - VI - 2) MOLECULAR CLONING OF A NOVEL PROCYCLIC FORM-SPECIFIC GENE (CDNA) FROM TRYPANOSOMA BRUCEI BRUCEI

SHIGERU OHSHIMA, TAKASHI SUZUKI, YOSHISADA YABU, MARIKO HATOH and NOBUO OHTA


Trypanosoma brucei brucei protozoa spreads between the mammal host and the tsetse fly vector in its lifecycle. In order to control Trypanosoma, we analyzed gene-expression profiles of blood stream and procyclic (insect) forms of T. b. brucei using the method of fluorescence differential display. About 12000 cDNAs were surveyed, and a total of 39 differentially regulated cDNA bands were identified. One markedly differential expressed cDNA, which was up-regulated in procyclic from, was selected and full length of which was obtained by 5’RACE method. The result showed that the gene is 3304bp encoding an ORF of 813 amino acids. Genbank database search revealed that this gene has 38% identity with Trypanosoma cruzi (T. cruzi) Cyclin 5 gene. T. cruzi Cyclin 5 was known to be bound to TzCRK1 (T. cruzi Cdc2p-related kinase 1) which is one of Cdk5 (cyclin dependent kinase). The function of T. cruzi Cyclin5 is still uncertain but presumed to play an important role(s) in controlling the cell. It is thus suggested that the novel procyclic form-specific gene (cDNA) from T. b. brucei also plays an important role in the process of the cell cycle similar to T. cruzi Cyclin5.

The result of RT-PCR demonstrated that the gene was not expressed in long slender blood stream form, while it was expressed in short stumpy (pre-adapted) form, which can change to procyclic form when the blood is sucked by tsetse fly. Therefore, it seems likely that this gene has an important function in lifecycle change of T. b. brucei from blood stream to procyclic forms. In order to clarify the function of the gene, RNAi-mediated gene knock down analysis is under way.

W - VI - 3) ANALYSIS OF LEISHMANIA AMASTIGOTE STAGE-SPECIFIC MOLECULES

CHIZU SANJOBA', TETSUHIKO SASAKI', YASUYUKI GOTO', TAKASHI ONODERA' and YOSHITSUGU MATSUMOTO

Dept. of Mol. Immunology, School of Agriculture and Life sciences, Univ. of Tokyo',
Dept. of Biological Sciences, Univ. of Tokyo'

A Leishmania protozoan differentiates from the promastigote to the amastigote during its digenetic life cycle between invertebrate and vertebrate hosts respectively. The promastigote can be easily cultivated in vitro. This has resulted in Leishmania promastigotes being investigated in a wide range of basic biological sciences and immunology on leishmaniasis. In contrast, in vitro cultivation of the amastigote stage is still a challenge. As a result, relatively little is known about amastigote stage-specific molecules. The proliferation of amastigotes in macrophages results in cell rupture and tissue damage, giving rise to the various symptoms associated with leishmaniasis. Thus, detailed investigation of amastigotes in teams of protein levels is crucial to understand the pathogenesis of leishmaniasis. Moreover, these studies will help to understand the survival mechanisms of amastigotes during infection within the mammalian host. The identification of amastigote stage-specific proteins was addressed by proteomic approaches using the technique of two-dimensional electrophoresis. The protein expression patterns of Leishmania major amastigotes purified from cutaneous lesions in experimentally infected Rag2 knock out mice (BALB/c) and cultured promastigotes were compared. The majority of protein spots occurred between 28 kDa and 94 kDa; at least 32 spots from amastigotes and 43 spots from promastigotes were clearly visualized on a Coomassie Brilliant Blue -stained gel. although 11 protein spots were found to be expressed by both amastigotes and promastigotes, and the remaining 21 protein spots were specific to amastigotes and 32 spots were promastigote specific. These results demonstrated that L. major expresses stage-specific proteins. Sequentially, to detect amastigote stage-specific antigens, proteins from promastigotes and amastigotes were analyzed by western blotting using the polyclonal antibodies from a rabbit immunized with homogenates of purified L. major amastigotes. Three specific spots of amastigotes were detected. Determination of amino acid sequences of these amastigote stage-specific antigens is now on going. These studies will contribute to the develop-
ment of immunological diagnosis or treatment, or effective vaccine against leishmaniasis with the new concept that amastigote stage-specific antigens should be targets for control measures.

**W - VI - 4)** THE QUANTIFICATION OF THE GENES INVOLVED NO PRODUCTION IN LEISHMANIA-INFECTED MACROPHAGE BY REAL-TIME PCR

YAMAMOTO, Y.

**W - VII - 1)** CHEMOTHERAPEUTIC APPROACH TO AFRICAN TRYPANOSOMIASIS: THE USE OF DIORGANOTIN AND ITS DERIVATIVES

NASIR MOHAMMED SHUAIBU1,2, HIROJI KANBARA1, TETSUO YANAGI1, AKITOYO ICHINOSE3, A. D. AMEH3, J. J. BONIRE2 and JONATHAN ANDREW NOK2

Department of protozoology, Institute of Tropical Medicine, Nagasaki University1, Biochemistry Department2, Chemistry Department3, Ahmadu Bello University, Zaria, Nigeria, Central Laboratory, Institute of Tropical Medicine, Nagasaki University4

African trypanosomiasis is an endemic disease in nature with recurrent outbreaks, affecting both human and his domestic livestock; with no vaccine at near sight. The disease is fatal if untreated and chemotherapy remains the most powerful strategy to date, albeit narrow therapeutic index. Despite the tremendous progresses made in the identification and characterisation of biochemical and molecular properties of trypanosomes, potential drug target to produce an “ideal trypanocide” proves elusive. Current drugs that are available are old, scarce, less effective, toxic and encounter parasite resistance. Therefore there is an urgent need for new drugs for the treatment of this disease. The development of *in vitro* drug screening assays has improved the search for compounds against pathogenic trypanosomes. The proliferation of trypanosomes is rapid and has been likened to some types of cancer cells and all the currently used drugs against human African trypanosomiasis (HAT) have anti-cancer effect or *vice versa*. DFMO was first developed as anti-cancer drug, but now has therapeutic activity against Gambian sleeping sickness, even in the late CNS-involved stage. Organometallics have also been reported to have both anti-cancer and anti-trypanosomal effects. To date only organoarsenic melarsoprol that cures all forms of HAT albeit undesirable and often fatal side effects. African Trypanosome are partially auxotrophic for lipids and are salvaged from the host environment for the synthesis of conventional membrane and GPI anchor.

In an attempt to explore the chemotherapeutic potential of organotin and to broaden the search for much needed trypanocide an approach was made against three species of *trypanosoma*, using organometallic dibutyltin dichloride and its fatty acid derivatives as antitrypanosomal agents *in vitro*. The compounds mediated the killing of the parasites with minimum inhibitory concentration (MIC) values ranging from 76.0-597.0 ng/ml, 53.2-597.0 ng/ml and 106.4-600.0 ng/ml for *Trypanosoma brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense*, respectively. Our observed selectivity indices of these compounds against murine-derived fibroblasts are not as high as the currently used trypanocides. These observations suggest the chemotherapeutic potentials of organotins and the approach could be useful in either developing new lead compounds or improve the use of the current trypanocide in use.
W - VII - 2) THE INDUCTION OF TNF-α AND iNOS EXPRESSION BY MACROPHAGE CELLS AGAINST ONO-4007 (A SYNTHETIC LIPID A DERIVATIVE), IFN-γ AND MEGLUMINE ANTIMONIATE IN VITRO STUDY

MOHAMMED ABUL KASEM KHAN1, MOTOYOSHI MARUNO1, NOOR MOHAMMAD KHASKHELY1, HIROSHI UEZATO1, EDUARDO A. GOMEZ LANDIRES3, YOSHIHISA HASHIGUCHI1 and SHIGEO NONAKA1

1) Department of Dermatology, Faculty of Medicine, University of the Ryukyus, Okinawa,
2) Department of Tropical Medicine, Catholic University OF Guayaquil, Guayaquil, Equador,
3) Department of Parasitology, Kochi Medical School, Kochi, Japan

Introduction: In leishmaniasis, TNF-α induces a lytic mechanism in protecting against Leishmania parasites through stimulating inducible nitric oxide synthase (iNOS), which catalyzes to nitric oxide (NO) by activated macrophage cells. The purpose of this study to clarify the antileishmanial action of ONO-4007 (a LPS derivative) or IFN-γ or meglumine antimoniate as a single or alternately in combination of ONO-4007 plus IFN-γ, induced by TNF-α and iNOS expression.

Material and Methods: 1) Promastigotes of L. major (MHOM/SU/73/5ASKH) and murine macrophage cell line, J774 were cultured in RPMI and DMEM medium respectively. 2) ELISA for TNF-α: Macrophages were cultured and infected with L. major promastigotes for 24 hrs. The drugs were administered as a single regime: ONO-4007 (0.01, 0.10 and 1.0 mg/ml), IFN-γ (1, 10 and 100U/ml), meglumine antimoniate (0.085, 8.5 and 850 µg/ml) and also in combination regime: ONO-4007+IFN-γ for 24 hrs. TNF-α in the supernatant was assessed at 6-24 hrs incubation by ELISA. 3) Immuno-histochemical study: Macrophages were cultured and incubated for 24 hrs with ONO-4007, IFN-γ, and also in combination of ONO-4007+IFN-γ. After 6, 12 and 24 hrs of incubation with these drugs, fixation of the cells were done with 100% ethanol alcohol and immunostaining performed by using primary antibody against iNOS (Wako, Japan).

Results and Conclusion: ONO-4007 alone and in combination of ONO-4007 plus IFN-γ, induced the significant higher level of TNF-α following 6-24 hrs of incubation at three different concentrations as compared to control. TNF-α production by infected-Mφ cells in response to IFN-γ and meglumine antimoniate as a single regime did not induce the significant higher level at three different concentrations as compared to contorol. Immunohistochemical observations revealed that the positive reactivity for iNOS expression were observed at 6 hrs of incubation and the intensity of the staining pattern gradually increased following 12 hrs and 24 hrs of incubation, in response to ONO-4007 and IFN-γ as a single and also in combination regime of ONO-4007+IFN-γ. From these observations, we suggest that ONO-4007 either alone or in combination of IFN-γ might act as an antileishmanial agent by inducing TNF-α and iNOS production by macrophage cells in vitro study. Meglumine antimoniate exerts its antileishmanial action through different mechanism but not involving the cytokine-immune pathway.

W - VII - 3) MARINE SPONGES; HOPEFUL SOURCES OF NEW ANTI-PROTOZOAN DRUGS

YASUYUKI GOTO1, YOICHI NAKAO2, CHIZU SANJOBA1, YUKO KATAKA1, SUKMAWATI BASUKI1, SHIN-ICHIRO KAWAZU1, TAKASHI ONODERA1 and YOSHITSUGU MATSUMOTO1

Department of Molecular Immunology, University of Tokyo1,
Department of Aquatic Natural Product Chemistry, University of Tokyo2,
Research Institute of International Medical Center of Japan3

In recent marine sponge has received much attention as a source of unique chemicals, which affect eukaryotic cells. Those chemicals include mycalolide B and jasplakinolide, which shows anti-tumor activities and effects on the structure of actin filaments, respectively. In this study we examined the anti-protozoan activities of extracts from various marine sponges. The lipophilic and hydrophilic layers from marine sponges were examined on the point of inhibition activities against Plasmodium falciparum and Leishmania amazonensis. Inhibition activities against P. falciparum
erythrocytic stages in vitro were examined by direct counting on Giemsa stained smears. For anti-leishmanial activities, L. amazonensis LV78 promastigotes transfected with enhanced green fluorescent protein (EGFP) gene were used. The fluorescence intensity of transfected promastigotes was in proportion to the number of promastigotes, indicating that this method could be the high throughput screening method. Among extracts from 93 marine sponges tested against P. falciparum, 8 lipophilic and 4 hydrophilic layers showed > 50% inhibition at the concentration of 1 µg/ml. For anti-leishmanial activities 5 lipophilic layers among those from 61 marine sponges showed > 50% inhibition at the concentration of µg/ml. Further fractionation of these lipophilic layers by HPLC, the 50% inhibition concentration (IC50) values of 4 fractions against P. falciparum and 4 fractions against L. amazonensis were < 100 ng/ml. These results suggest that marine sponges are hopeful sources of new anti-protozoan drugs.

W - VII - 4) COMBINATION EFFECTS OF CHLOROQUINE WITH FEBRIFUGINE / ISOFEBRIFUGINE MIXTURE AGAINST CHLOROQUINE-RESISTANT PLASMODIUM BERGHEI NK65 IN ICR MICE

AKIRA ISHIH1, TOHRU SUZUKI1, MACHIKO WATANABE1, TOSHIO MIYASE2, MAMoru TERADA1

Department of Parasitology, Hamamatsu University School of Medicine1,
Department of Pharmacognosy, School of Pharmaceutical Science, University of Shizuoka2

Of strategies on controlling malaria which is one of the major health problems in the world, chemotherapy is the primary defense against malaria. Under world-wide emergence of chloroquine-or multiple drug-resistant variants of Plasmodium falciparum, new approaches to treatment of malaria are urgently required as well as a development of new effective drugs. Recently, several reports have been published in combination therapy. In this study, we report our results on combination of chloroquine (CQ) with febrifugine/ isofebrifugine mixture (FI) in ICR- P. berghei NK65 model. The alkaloid fraction was prepared from the dried leaves of H. macrophylla var. Otaksa using a porous polymer gel (Mitsubishi Diaion HP-20) column and a cation exchanger (Amberlyst 15) column. Outbred male ICR mice, 7 weeks old, were infected intraperitoneally with 1x10^7 P. berghei NK65-parasitized erythrocytes. 1) From day 4 after injection of parasitized erythrocytes, mice were treated orally with a single dose of 40 mg/kg of CQ base, a two-day dosage of 20 or 40 mg/kg of CQ base or a three-day dosage of 20 mg/kg of CQ base in the treated group. Distilled water was given in the untreated, infected mice. Mice in the untreated control showed a progressively increasing parasitemia leading to death. A treatment of CQ alone showed little effect against P. berghei NK65 infection, and all mice died up to day 17 with an increasing parasitemia. 2) A four-day dosage of 1 mg/kg, twice a day, of FI alone showed a little effect, but all mice died up to day 27. All mice treated with FI showed low parasitemia levels during a drug administration and following a few days, but then malaria parasites increased in the bloodstream of the treated mice until death. On the other hand, mice treated with CQ plus FI survived during the experiment. Malaria parasites in the mice given CQ plus FI decreased on day 6 and then could not be detected by a microscopic examination during observation period. Studies are in progress to clarify the mechanisms of combination effects of CQ plus FI against the parasite.
PURPOSE: To determine in an active survey the presence of mucocutaneous leishmaniasis (MCL) in persons living at north east of the Amazonian region of Ecuador.

INTRODUCTION: MCL is the most serious and destructive clinical form of American cutaneous leishmaniasis. Figures of skin lesions facial deformities have been represented on pre-Inca pottery from Ecuador, Peru and Colombia. Although MCL was reported since 1924 and is believed to be endemic in the Amazonian region, relatively little active research has been done. Data reported in Ecuadorean medical journals during 1920 to 1987 showed 260 cases, of these 18 (6.9%) were MCL.

STUDY AREA, PATIENTS & METHODS: The study was carried out in the provinces of Sucumbios and Orellana. Individuals suspected to have MCL were clinically and parasitologically examined using Giemsa-stained smears, culture of aspirated, PCR and histopathologic study of skin biopsy. Sandfly collections were done by protected human bait. Itraconazole was the drug used to treat all patients.

RESULTS: 13 patients were found positive. The age ranged from 22 to 67 years. All patients have got lesions in nasal mucosa, septum and turbinate, 5 in upper lip and 4 in pharynx. Lesions in early stages encountered were erithema, bleeding and crusts, nasal infiltration, nodules and granulomas in the septum. Large evolution determined septal perforation, swelling of upper lip, protuberant nose and disphonia. 12 of 13 had evidence of previous scars. 7 antrophophilic Lutzomyia could be identified.

DISCUSSION & CONCLUSIONS The progressive inflammatory destruction seen in the more advanced cases resulted in septal perforation (7 cases), and invasion of the larynx causing dysphonia (4 cases). 7 (53.8%) were classified as having severe MCL. Severe disease might follow a long period of evolution and/or be attributed to the virulence of L.(V.) braziliensis. None of the subjects had evidence of active CL. These findings are in agreement with previous observations but contrasts with those reported from Colombia. These differences may reflect the parasite species and ecological location. This active search favors the description of the lesions in early and late stages. This report emphasises the importance of an active search in the endemic communities for early diagnosis and treatment to prevent irreversible disfigurement.
occurrence of circulating visit to each subvillage, and revealed that most of malaria cases appeared in forested and hilly areas and a few cases in coastal subvillages located at the northern part of the project area. In forested, hilly areas malaria cases were found constantly through the year. Since in coastal areas malaria usually shows a seasonal epidemic, there still remains a possibility of a sudden occurrence of malaria epidemic although only a few cases were found at the moment. On the map of two subvillages for the regular survey, malaria cases were plotted. No biased distribution was not demonstrated. The entomological team demonstrated that the main vector in the forested area was Anopheles balabacensis and that in the coastal was An. sundicus. The parasitological team examined cerebral malaria cases at the Mataram hospital and found that most of the cases were from forested and hilly areas. This indicated that malaria information which we had obtained previously was biased by the old preconception. Although we began the first trial of distribution of impregnated mosquito nets and residual indoor spraying in selected subvillages, we suppose that these trials would be effective in the coast but not in the forest judging from the results of the entomological survey.

**B - 3) BABESIA SP. AND OTHER HEMOPARASITES IDENTIFIED IN WILD RATS IN CHIANG MAI AND MAE HONG SON PROVINCE, THAILAND**

ANCHALEE DANTRAKOOL¹, ATSUKO KAWAI¹, PRADYA SOMBOON², ATSUKO SAITO-ITO¹

Section of Parasitology, Graduate School of Medicine, Kobe University¹, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand²

Wild rodents are thought to be the most important reservoir hosts of zoonotic infectious diseases especially when a tick is involved as a vector. Human babesiosis is a typical case of emerging tick-transmitted parasitic diseases induced by wild rodent reservoirs. Thus, human babesiosis, which is mostly caused by rodent babesias, Babesia microti, has occurred mainly in the United States, and a few sporadic cases have recently emerged in the other countries including Japan. In order to infer the possibility of the emergence or latent existence of human babesiosis in the malaria endemic area, we started in 2000 to investigate the prevalence of Babesia infection in wild rodents in Chiang Mai and Mae Hong Son Province, Thailand and partly reported elsewhere. Here, we report the results of the consecutive investigation and analysis regarding Babesia infection as well as the detection of other hemoparasites in wild rats in the area.

During 2000 to 2002, the epizootiologic surveys for wild rats were carried out at several sites in Chiang Mai and Mae Hong Son Province, Thailand. In total 47 wild rats, including 30 Bandicota indica and 17 Rattus exulans, were caught. The blood smears showed that 17 of 30 B. indica were parasitized by Babesia sp. (parasitemia 0.03-11.02%) but not in any of 17 R. exulans. All of Babesia-positive B. indica were captured in Chiang Mai but not in Mae Hong Son Province. Twelve of 17 Babesia-positive B. indica were infested with ticks of Haemaphysalis spp.. No other kinds of ticks were identified on any of captured wild rats. All of the ss-rDNA sequences of 6 Babesia isolates from different rats were completely identical without regard to the site and the year for capture of rats. The phylogenetic analysis based on the sequence of ss-rDNA showed that the Thai Babesia sp. is most closely related with canine babesias, B. canis and B. gibsoni, but not so with B. microti. Two dogs kept near the site where the prevalence of Babesia infection in wild rats was high were found negative with Babesia parasites by PCR as well as blood smears. Regarding other hemoparasites, two Babesia-positive B. indica showed the simultaneous infection of Hepatozoon-like parasites (gamonts) in leukocytes. Hepatozoon is also known to be transmitted by ticks. The ss-rDNA sequence analysis supported that the parasite is most closely related with Hepatozoon sp.. On the other hand, Trypanosoma sp. was identified only in R. exulans (3/17), but not any of B. indica. Although human babesial infection has not been reported in Thailand, the risk of zoonotic babesial infection cannot be denied at present. Indeed, many ticks bites are noticed for human as well as for domestic dogs in the area. Serologic survey using the Thai babesial infection is hold among human population in the area.
**B - 4)**  **ADULT BLACK FLIES ATTRACTED TO HUMANS AND WATER BUFFALOES, AND NATURAL INFECTIONS WITH ONCHOCERCA LARVAE IN CHIANG MAI, IN NORTHERN THAILAND**

HIROYUKI TAKAOKA¹, CHHARU AOKI¹, MASAKO FUKUKA¹, WEJ CHOCHOTE² AND ODILE BAIN³

Department of Infectious Diseases, Oita Medical University¹, Department of Parasitology, Chiang Mai University², Prasitologie comparée et Modèles expérimentaux, associé à l’INSERM (U567), et Ecole paratique des Hautes Etudes, Muséum National d’Histoire Naturelle³

Several Simulium species were investigated as to their biting habits and natural infections with filarial larvae at Ban Pan Fan, Chiang Mai Province, in northern Thailand. Female adult flies landing on or flighting around a human and a water buffalo were collected during the daytime from 06.00 to 19.00 hours on 22 June 2001. As a result, 217 S. nodosum, 86 S. asakoae and 2 S. nigrogilvum were obtained from a human attractant, and 416 S. nodosum, 25 S. nakhonense, 16 S. asakoae, 4 S. fenestratum and 2 S. nigrogilvum, from a water buffalo. The blood-feeding was confirmed only for S. nodosum and S. nigrogilvum on humans, and for S. nodosum and S. nakhonense on water buffalos. Dissections of these simuliids showed that S. nodosum was naturally infected with developing filarial larvae. Two types of microfilariae were distinguished but only one type of infective larvae. These larvae resembled Onchocerca suzukii, a parasite from a wild Japanese bovid, suggesting that an unknown Onchocerca species from ruminants was transmitted in Thailand. Infection rates with all stages of larvae and third-stage larvae were 2.3% (14/608) and 1.0% (6/608), respectively. This is the first report of natural infections of black flies with Onchocerca larvae in Southeast Asia, and the involved black-fly species is anthropo and zoophilic.

**B - 5)**  **SEASONAL CHANGES OF WILD ANOPHELINE MOSQUITOES AROUND JICHI MEDICAL SCHOOL**

HIROYUKI MATSUOKA, MEIJI ARAI, SHIGETO YOSHIDA, AKIRA ISHII

Department of Medical Zoology, Jichi Medical School

**Abstract**

We have collected wild mosquitoes around Jichi Medical School, Tochigi Prefecture, for two years (2001-2002). A light trap was hanged overnightly in a cattle hut 2 km far from Jichi Medical School, and the number of mosquitoes collected was counted next day. Mosquito species collected were Anopheles sinensis, Culex tritaeniorhynchus, Culex pipiens pallens etc.. An. sinensis, which can transmit vivax malaria, was collected from June to September, in particular, middle July to beginning of August. On the peak day, more than 1800 of An. sinensis female mosquitoes were collected. Almost all An. sinensis had fed blood. Cx. tritaeniorhynchus, which can transmit Japanese encephalitis, made a peak, more than 1600 per night, in August. Three weeks interval was observed between peaks of An. sinensis and Cx. tritaeniorhynchus. Both mosquito species might feed on cattle and oviposit in paddy fields several hundred meters far from the cattle hut. In winter, no adult mosquitoes were collected from the cattle hut. We should notice that these vector mosquitoes still live together with us in Japan. We will follow these mosquitoes’ number as the information for the future.
B - 6) A NOVEL GT-MISMATCH BINDING PROTEIN POSSIBLY INVOLVED IN BASE EXCISION REPAIR OF THERMALLY INDUCED MATAION

Maki Yahiro¹, Yoshito Fujii¹, Shuji Yonei¹, and Michio Nakamura¹

¹Department of Host-Defense Biochemistry, Institute of Tropical Medicine, Nagasaki Universtity, and ²Laboratory of Radiation Biology, Graduate school of Science, Kyoto University

Watson-Crick-type base pairs are extremely strict in genomic DNA because of high specificity of polymerase activities and error-correcting machineries. Even nonreplicating DNA is exposed to error-prone stresses such as reactive oxygen species, radiation and high temperatures that modify bases or cut backbone DNA strands. Among mismatches found in DNA, a G/T mispairing is most common because cytosine is frequently deaminated after an enzymatic methylation. We report here a serendipitously discovered novel G-to-T-mismatch binding protein (nGTBP).

This protein bound to a CYBB promoter-originated 23-mer heteroduplex with a G/T mismatch at bp-177. None of heteroduplexes with the rest 15 possible base pairs at bp-177 bound to nGTBP. Similar heteroduplexes with G/T or T/G at different bp positions failed to recruit nGTBP. DNA heteroduplex-nGTBP complex was not disturbed by an MSH6 consensus oligomer nor anti-MSH6 antibody on an electrophoresis mobility shift assay. In addition, the nGTBP was detected in MSH6 deficient cell lines. The protein minimally required a middle 14-mer heteroduplex of the original 23-mer and TRTGNB as the core sequence with the G mispairing with T. Neither oxidized T [thymineglycol] nor oxidized G [8-oxoguanine] at bp-177 of 14-mer homoduplex made the duplex recruit the nGTBP. Deaminated A [hypoxanthine] and deaminated C [uracil], however, made the duplex bind to the nGTBP. These results suggest that the nGTBP primarily recognizes deaminated “matched” pairs. Deaminations occur by spontaneous hydrolysis that is increased by high temperature. Accordingly this nGTBP may be involved in the repair of thermally injured deoxynucleotides.

B - 7) MITOCHONDRIAL QUINOL-FUMARATE REDUCTASE (COMPLEX 辋 FROM ASCARIS SUUM AS A TARGET FOR CHEMOTHERAPY

Arihiro Osanai¹, Noriko Shinjyo¹, Hiroko Miyadera¹, Ting Wang¹, Hisako Amino¹, Shigeharu Harada², and Kiyoshi Kita¹

¹Department of Biomedical Chemistry, School of Medicine, The University of Tokyo¹, ²Department of Protein Structural Biology, School of Pharmaceutical Sciences, The University of Tokyo²

Ascaris suum, a parasitic nematode, lives in host small intestine, where oxygen tension is limited and develops a unique anaerobic energy generation system. In this system called NADH fumarate reductase system, mitochondrial complex 辋 catalyzes fumarate reduction and functions as a terminal oxidase. The difference between A. suum and mammalian complex 辋 that functions as succinate dehydrogenase gives us an idea that complex 辋 must be a promising target for chemotherapy. In this study, we purified A. suum complex 辋 to almost homogeneity and performed enzymatical studies and crystallization.

First, we established a new method of mitochondria preparation from A. suum muscle and we could prepare 500 mg protein of mitochondria from 500 g (about 400 nematodes) muscle. Second, we performed 2 kinds of anion-exchange chromatography and could purify about 7.5 mg of complex 辋 from 1000 mg protein of mitochondria.

In enzymatical analysis, we showed that A. suum complex 辋 had higher affinity to fumarate than bovine complex 辋 and that A. suum complex 辋 could function as fumarate reductase by this high affinity to fumarate. Some inhibitors were tested and we showed that TTFA and carboxin, the inhibitors of bovine complex 辋, did not inhibit A. suum complex 辋 and this suggests that the structure of inhibition site must be different between A. suum and bovine complex 辋. So flutolanil can be a promising lead compound of anthelmintics.

In trials of crystallization, we could obtain the crystal with the size of 30 micrometer reproducibly. Stabilization of A. suum complex 辋 activity by additon of 1 mM malo-
nate and screening of wide range of detergents led to the crystal growth of *A. suum* complex urtles. Now the X-ray diffraction study is undergoing.

This study shows that complex urtles is a promising target for chemotherapy and is a first step for development of new anti-parasitic drugs.

**B - 8) CHEMOTACTIC FACTORS FOR LEUKOCYTES FROM EGGS OF SCHISTOSOMA MEKONGI**

**MAKOTO OWHASHI**1, **JUN MATSUMOTO**2, **MASASHI KIRINOKI**3, **VIROJ KITKOON**4, **HAIJME MASTUDA**5

1Department of Life Science, Faculty of Integrated Arts and Sciences, Tokushima University, 2Dokkyo University, School of Medicine, 3Mahidol University, Faculty of Tropical Medicine

We previously showed that soluble extract of eggs (SEA) of *Schistosoma-japonicum* possesses intense chemotactic activities for eosinophils or neutrophils, and pointed out a contribution of the granulomatous lesions around deposited eggs in the liver. *Schistosoma mekongi* is one of the newly recognized species of *Schistosoma* that is closely related to *Schistosoma japonicum*. However, little information is available about the pathogenesis of *S. mekongi* infection.

In the present study, we examined leukocyte chemotactic activity of SEA of *S. mekongi* using multi-well microchemotaxis chambers equipped with Millipore membrane filters with a pore size of 3 μm. SEA of *S. japonicum* was used as a positive control. *S. mekongi* SEA showed lower eosinophil chemotactic activity than *S. japonicum* SEA. In contrast, *S. mekongi* SEA showed more intense neutrophil chemotactic activity than *S. japonicum* SEA. Western blot analysis revealed that *S. mekongi* SEA generally showed a similar antigenecity with *S. japonicum* SEA, whereas *S. mekongi* SEA showed lower affinity to an antibody against the high-molecular weight ECF purified from *S. japonicum* SEA. Toluene-extractable fraction of *S. mekongi* SEA showed comparable chemotactic activities with that of *S. japonicum* SEA, whereas toluene-nonextractable fraction of *S. mekongi* SEA showed lower chemotactic activity than that of *S. japonicum* SEA did. These results indicate that a defect of glycosilation of the high molecular weight components of SEA resulted in the lower grade of eosinophil chemotactic activity of *S. mekongi* SEA than *S. japonicum* SEA.

**B - 9) CHARACTERIZATION OF RECOMBINANT FRAGMENTS OF THE 150-KDA LECTIN (IGL) OF ENTAMOEBA HISTOLYTICA**

**HIROSHI TACHIBANA**1, **XUN-JIA CHENG**2,3 AND **YOSHIMASA KANEDA**1

1Department of Infectious Diseases, Tokai University School of Medicine, Japan, 2Department of Medical Microbiology, Medical Center, Fudan University, China

Carbohydrate-protein interactions initiate the contact-dependent cytotoxicity for which *Entamoeba histolytica* was named. It is well known that the 260-kDa galactose (gal)- and *N*-acetyl-D-galactosamine (GalNAc)-inhibitable surface lectin of the ameba mediates adherence to human colonic mucins, colonic epithelial cells, neutrophils, and erythrocytes. Recently, we identified another Gal/GalNAc lectin, a 150-kDa surface antigen recognized by monoclonal antibodies (mAbs). The mAbs inhibited amebic adherence to erythrocytes and Chinese hamster ovary (CHO) cells, erythrophagocytosis, and cytoxicity to CHO cells. The 150-kDa lectin, named as intermediate subunit (Igl), is a cysteine-rich protein consisting of 1101 amino acids and containing multiple CXXC motifs in amino acid sequences. In the present study, various lengths of recombinant fragments of Igl were prepared in *Escherichia coli* and the reactivity of the mAbs to the fragments was examined for epitope localization. Two of four adherence-inhibitory mAbs reacted with the recombinant protein of full length but with none of the partial fragments, demonstrating that conformation of the protein is important. The epitopes of the two other inhibitory mAbs were localized in the parts of amino acids 603-753 and amino acids 989-1088. These parts may have the carbohydrate recognition sites and could be vac-
Protein degradation by ubiquitin-proteasome pathway plays an essential role for a wide variety of cellular processes in eukaryotic cells. Studies with selective proteasome inhibitors such as lactacystin have demonstrated possible contribution of protein degradation by this pathway in cellular processes. Therefore, we examined whether proteasome inhibitors affect growth and encystation as well as excystation of *Entamoeba*. Three proteasome inhibitors, lactacystin, clasto-lactacystin β-lactone, and MG-132, were used for *Entamoeba histolytica* and *E.invadens*. All these drugs inhibited *E. histolytica* growth in a concentration-dependent manner, while lactacystin was most potent for the inhibition and MG-132 showed the inhibitory effect only at higher concentrations. *E. invadens* was more resistant to these drugs than *E. histolytica*. Encystation of *E. invadens* was also inhibited by these inhibitors and was more sensitive to the drugs than the growth, where the β-lactone was most potent for inhibition of encystation. The inhibitory effect of lactacystin and the β-lactone on encystation was slightly or little abrogated by the removal of the drug, respectively. Multinucleation occurred in *E. histolytica* trophozoites treated with these drugs, being most marked by lactacystin. Electron microscopy revealed that treatment of *E. histolytica* trophozoites with lactacystin led to an increase in the number of cells with many glycogen granules in the cytoplasm. Lactacystin, β-lactone and MG-132 had no or little effect on the excystation and metacystic development of *E. invadens*. These results show that proteasome function plays an important role for *Entamoeba* growth and encystation, but has no obvious effect on the excystation and metacystic development.

Protein farnesylation, which is catalysed by farnesyltransferase (FT), is one of the post-translational lipid modification of proteins such as low molecular weight GTPase Ras, and thereby enables Ras to attach membranes and interact with other proteins. To elucidate significance of protein farnesylation in *Entamoeba histolytica* and to evaluate its FT as a target for chemotherapy, we have started biochemical characterization of FT of this parasite. We obtained cDNA encoding α-and β-subunits of FT (FTα and β) of *E. histolytica* by PCR amplification using primers designed on the fragments of corresponding genes found in the TIGR *E. histolytica* genome database. The genes of *E. histolytica* FTα and FTβ encoded 298 and 375 amino acids, respectively, and showed 24-36% positional identity with those of human, *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, and *Trypanosoma brucei*. The coding regions and the ribosome binding sites between them were cloned in tandem in pQE31, and expressed in *E.coli*. The 38 kDa and 43 kDa proteins corresponding FTα and FTβ, respectively, were identified by SDS-PAGE. The recombinant *E.histo-
lytica FT showed a FT activity against wild type human recombinant Ras (-CVLS) but not against mutant human Ras (-CVLL). We then prepared 4 recombinant E.histolytica Ras homologues (-CIMF, -CELL, -CSVV, -CVVA) to examine their substrate activities. Among them, only Ras (-CVVA) showed the substrate activity against the recombinant E. histolytica FT, suggesting a difference in substrate specificity between human and E. histolytica FT. These results suggest that E. histolytica FT may become a possible target for chemotherapy.

B - 12) RECENT SITUATION OF DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER IN THE PHILIPPINES (1995 - 2001)

RONALD R.MATIAS1, SHINGO INOUE1,4, MARIA T. D. CINCO1, CORAZON C. BUERANO1,3, KAZUNORI OISHI5, KOUICHI MORITA1, Futoshi HASEBE1, JHOE A. R. ALFON1, DEU J. M. CRUZ1, MARY ANN. PANCHO1, MA LUISA G. DAROY1, EFREN M. DIMAAANO1, CELIA C. CARLOS1, AKIRA IGARASHI1, FILIPINAS F. NATIVIDAD1

1Research & Biotechnology Division, 2Department of Pediatrics, St. Luke’s Medical Center, Philippines, 3Institute of Biology, University of the Philippines, Diliman, Philippines, 4Department of Virology, 5Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 6Pavilion 1, San Lazaro Hospital, Philippines

[Introduction] Dengue fever (DF) and dengue hemorrhagic fever (DHF) cases were reported approximately 13,000 every year and its mortality rate was 1% among children and adolescent in the Philippines. The Research and Biotechnology Division, St. Luke’s Medical Center and the Institute of Tropical Medicine, Nagasaki University have done collaborative study on dengue for 7 years (1995-2001) in Metro Manila. To know the impact of DF and DHF in the Philippines, we conducted virological, serological and clinical surveillance using blood samples and clinical records of dengue suspected / clinically diagnosed patients.

[Materials and Methods] Using 3,564 blood samples, virus isolation using C6/36 mosquito cells and RT-PCR were performed as virological examinations. Hemagglutination inhibition test (HI test), IgM-capture ELISA and IgG indirect ELISA were done as serological examinations. 

[Results and Discussion] The peak season of dengue virus infection was from July to November. The dengue outbreak occurred every three years that were in 1995, 1998 and 2001. Dengue confirmed cases were 53% of the examined cases and approximately 80% of them were dengue secondary infection examined by HI test and IgG indirect ELISA.

The major serotypes of isolates were DEN2 and 3 in 1995 but it gradually changed to DEN 2 and 1 by 2001. The phylogenetic analysis of DNE2 envelope region indicated the DEN2 virus local strains circulate within the Philippines. The severity of clinical symptoms based on WHO criteria could classify dengue as DHF grade 2 (38%), DF (31%) and Grade 3 (10%) among 863 dengue laboratory confirmed cases.

B - 13) A MECHANISM OF THROMBOCYTOPENIA DURING THE ACUTE PHASE OF DENGUE VIRUS INFECTION: INVOLVEMENT OF PLATELET-ASSOCIATED IGG AND ANTI-DENGUE VIRUS IGG

OISHI K1, INOUE S2, HASEBE F2, KUMATORI A3, MORITA K2, NAGATAKE T1, ALFON JAR4, CRUZ DJM4, CINCO MTD4, MATIAS RR1, ALERA TP4, DIMAAANO EM3, NATIVIDAD FP4


Although the public health impact of dengue is rapidly increasing, the mechanism of thrombocytopenia in this disease remains unknown. To elucidate this mechanism, the relationship between platelet-associated IgG (PAIgG) and platelet count in 83 patients with secondary dengue virus infection was investigated in a prospective-hospital-based study. A significant increase in PAIgG levels as well as a significant decrease in platelet count were found in all cases of secondary infection, compared with 57 healthy volunteers. Only two of the 83 patients with secondary dengue
virus infection were positive for anti-platelet IgG in plasma. The PAIgG levels in 53 patients with a platelet count of less than 100x10^11/µL were significantly higher than those in 30 patients with platelet counts greater than 100x10^11/µL or those from healthy volunteers. A significant inverse correlation between the two parameters was found. The low baseline platelet counts during the acute phase in 12 patients significantly increased during the convalescent phase, while the increased PAIgG levels of the acute phase in these patients significantly decreased during the convalescent phase. Involvement of anti-dengue virus IgG was also shown in platelets from 8 patients.

These findings suggest a pivotal role of PAIgG formation, involving anti-dengue virus IgG, in the induction of transient thrombocytopenia during the acute phase of secondary dengue virus infection.

B - 14) EVALUATION OF A HEPATITIS B VACCINATION IN CHIANG MAI, THAILAND

KAN TORIYAMA¹, MASACHIKI SENBA¹, PRAPAN JUTAVIJITTUM², AND AMNAT YOUSUKH²

Department of Pathology, Institute of Tropical Medicine, Nagasaki University¹,
Department of Pathology, Faculty of Medicine, Chiang Mai University²

In 1992, the Government of Thailand started a nationwide vaccination program for mother-to-infant transmission of hepatitis B virus (HBV), following a pilot program in Chiang Mai and Chon Buri. We report the long time efficacy of this vaccination program in Chiang Mai. Ninety-three percent of the urban and 60% of rural children had received adequate vaccination in the 4 to 6 year-old age group. The prevalence of HBsAg and anti-HBs positivity was 0.9 and 42.8% in the children who were adequately vaccinated, compared to 7.2 and 24.6% in the children who were not or inadequately vaccinated, respectively. The prevalence of HBsAg and anti-HBs positivity of the adequately vaccinated children was 1.1 and 33.8% in urban areas and 0.5 and 28.2% in rural areas, respectively. These findings indicated that the program has a certain level of efficacy, however it can be hardly said to be sufficient, comparing with the result of Japan. Several factors such as the commercial source of the vaccines, improper transportation and storage systems and insufficient methods of the administration might reduce the effectiveness of vaccination.

B - 15) ACUTE RESPIRATORY INFECTIONS (ARI) AMONG CHILDREN IN VIETNAM: DETERMINATION OF BACTERIAL ETIOLOGY AND ANTIBIOTIC RESISTANCE OF PATHOGENS

RUSIZOKA OSI¹, WATANABE K¹, CHEN M¹, NGUYET NT³, THI NT³, DUNG NT³, ANH NTC³, HUONG PLT³, ANH DD³, Oishi K¹, Nagatake T¹.

Dept of Intern Med¹, Inst of Trop Med, Nagasaki Univ. National Inst of Hyg and Epidemiol², National Institute of Pediatrics³, Bach Mai Hospital¹, Hanoi, Vietnam

Acute respiratory infections (ARI) are common causes of morbidity and mortality in tropical and developing countries. This study was designed to determine whether a quantitative culture method employing bronchial secretion obtained by nasopharyngeal swab is useful for determination of causative bacterial pathogens of ARI among children, and to determine the antibiotic susceptibility of major bacterial pathogens.

We enrolled 102 patients with ARI and 43 control patients without ARI. A chest radiograph was taken and bronchial secretion was obtained from each patient by nasopharyngeal swab, and the quantitative culture of bronchial secretion was done.

The isolation rate of bacterial pathogens was 51.0% (25/49) in patients with bacterial pneumonia (BP), 30.2% (16/53) in patients with acute bronchitis (AB) and 18.6% (8/43) in control (CT) patients, respectively. The isolation rate in BP, but not in AB, was significantly higher than that in CT. Major pathogens isolated were S.pneumoniae (n=12) and H.influenzae (n=11). Major bacterial pathogens of 102 patients of ARI were S.pn (45.5%) and H.inf (36.3%), respectively. We determined the MICs of them and also ex-
examined drug resistant genes of these strains by PCR. A very high rate of penicillin-resistant \textit{S.pn} (85\%) and ß-lactamase producing \textit{H.inf} (70\%). Most of PRSP strains had penicillin-binding protein (PBP) mutations in all of \textit{pbpla}, \textit{pbp2x} and \textit{pbp2b}, and were belong to serotype 19F or 23F.

Our data suggest a quantitative culture method using bronchial secretion is a useful tool for the bacterial diagnosis of BP in pediatric patients, and demonstrate a high rate of ß-lactam antibiotic resistance in major bacterial pathogens of pediatric patients of ARI in Vietnam.

\textbf{B - 16) A TRIAL OF NOSOCOMIAL INFECTION CONTROL IN THE BACH MAI HOSPITAL PROJECT FOR FUNCTIONAL ENHANCEMENT IN VIETNAM}

\textbf{HIROSHI OHARA$^1$, NGUYEN VIET HUNG$^2$}

Bureau of International Cooperation$^1$, International Medical Center, Department of Nosocomial Infection Control, Bach Mai Hospital$^2$

A technical cooperation project has been implemented in Bach Mai Hospital in Vietnam since January 2000 with the purpose of upgrading the quality of medical care service in the hospital and disseminating the benefit of better medical care to wide areas. In this project, nosocomial infection control was integrated in the program under the recognition that effective control is indispensable to improve the quality of medical care. This is the first trial as a hospital project in developing countries. In this study, the process of the introduction of nosocomial infection control was summarized aiming to realize better control in the developing countries.

The activities of nosocomial infection control in the project are as follows:

1. Setting up of Nosocomial Infection Control Department and allocation of staff
2. Supply of essential equipment and training of the staff
3. Inspection of current situation of nosocomial infection control in some hospitals
4. Making out a manual, teaching materials, pamphlets, posters, etc.
5. Organizing training courses and evaluation
6. Carrying out a fact-finding study for 1,266 in-patients in Bach Mai Hospital

After the start of the project consciousness of nosocomial infection control among hospital staff is increasing and control system is being created. The average score of the tests conducted after the training courses showed the increase of 29.4 compared with pre-tests, and the trainees had high satisfaction. The fact-finding study revealed infection rate with 5.77\% (73/1,266). As causative agents, \textit{Pseudomonas aeruginosa}, \textit{Acinetobacter baumanii}, \textit{Escherichia coli}, \textit{Enterococcus}, \textit{Klebsiella pneumoniae} revealed high frequency. 19 out of 73 cases were regarded as infections associated with surgical procedures.

Nosocomial infection rate in developing countries is high and effective control method has been increasingly requested. It should be stressed the importance of basic sanitary procedures such as hand washing. Enlightenment using manuals, teaching materials, and conducting training courses are also effective. In addition, appropriate use of antibiotics based on the results of monitoring causative agents is desired.
We found a case of keratitis caused by a species of microsporidium which was first isolated from the case. We successfully established an in vitro culture technique for the microsporidium, and we gathered from the morphology by transmission electron microscopy (TEM) that the species was *Trachipleistophora anthropophthera* in all likelihood.

The specimen of corneal biopsy was obtained from a 42 years old HIV-infected patient at King Chulalongkon Memorial Hospital, in Thailand. The man suffered from chronic keratitis in his left eye for more than 6 months. Several corneal scrapes stained for bacterial, fungal and viral agents were all negative. The corneal specimen was inoculated into a 25 cm² culture flask with fibroblasts derived from newborn mouse brain, and maintained with MEM culture medium supplemented with 5% fetal bovine serum and 5% newborn calf serum at 26°C, 29°C or 37°C. The microorganism could multiply at the same proliferation speed at the three different temperatures. Many oval-shaped spore, which was compatible with microsporidium, was detected under a light microscope after the smear of foci in fibroblasts was stained with chromotrope 2R or Calcofluor White M2R. The cell cultures were periodically scraped, fixed in glutaraldehyde, and examined with transmission and scanning electron microscope. Bisporous and multisporous vacuole-like sporophorous vesicles were observed simultaneously in foci of the microsporidia under TEM. They proved the species that was identified as *T. anthropophthera*, not as *T. hominis*.

To investigate the course of the infection, the spores from cultures were inoculated into SCID mice intraperitoneally (ip) or intramuscularly (ip). The mice were sacrificed to look for which tissue and organs were infected with the microsporidia. We initiated the tissue cultures of the SCID mice to isolate spores from each cultivation. We examined the stump preparation and pathological sections, and detected DNA of the microsporidium by PCR in each organ. In the im mice, the infection site was limited in the original part. In the ip mice, the spores became spread into the general body except the lung, probably through lymphatic circulation. These findings clearly indicate that microsporidia can be infectious into almost organs of a SCID mouse. Immuno-histological assay will be helpful to confirm accurately further which tissue and organs are infected.

Since intraperitoneal and intramuscular inoculation is artificial, we will infect by different transmission routes (ocular, intranasal and oral inoculation) into SCID mice in the near future.
C - 01) ANALYSIS OF ANTIGEN-SPECIFIC IGG SUBCLASSES IN SERUM AND URINE SAMPLES FROM BANCROFTIAN FILARIASIS PATIENTS

XU-GUAN QIU¹, MAKOTO ITOH¹, MIRANI V. WEERASOORIYA², MOHAMMAD ZAHIDUL ISLAM³, AND EISAKU KIMURA⁴

Dept. Parasitol., Aichi Medical Univ.¹, Dept. Parasitol., Univ. of Ruhuna, Sri Lanka²

A sensitive and specific urine ELISA, which detects filarial antigen-specific antibodies, is suitable for field studies. However, basic information on the immunoglobulins and total protein in urine and their correlation to those in serum has not been well studied. In this study, antigen-specific IgG subclasses are compared using paired serum and urine samples from bancroftian filariasis patients.

Seventy-six paired serum and urine samples from the antigen positives were used in this study. They were collected from residents in Matara, Sri Lanka, an endemic area of bancroftian filariasis. Blood sample, 100 µl, was taken on a filter paper strip. Collected urine samples were added with sodium azide. Bancroftian filaria-derived antigen was detected in serum samples eluted from the filter paper using an antigen detection kit (Trop-Ag W. bancrofti). Anti-Brugia pahangi female adult worm specific IgG subclasses were measured by ELISA. Serially diluted standard serum samples were applied to each ELISA plate to make a standard curve for each subclass. Each IgG subclass was shown with unit calculated from the standard curve. Urine samples were used directly and serum samples were diluted 5000 times for IgG1 and IgG4, and 100 times for IgG2 and IgG3. Protein levels in urine samples were measured using Protein Assay (Bio Rad).

Both IgG1 and IgG4 to B. pahangi antigens were detected in serum and urine samples: their levels in serum and urine samples were correlated well (r=0.67, P<0.0001 and r=0.73, P<0.0001, respectively). Strong correlation between the IgG1 and IgG4 levels both in serum and urine samples were also observed (r=0.71, P<0.0001 and r=0.70, P<0.0001, respectively). Both IgG2 and IgG3 subclasses to the antigens in urine samples were low and some of them were not detectable. Very few part (0.1~0.001%) of serum IgG1 and IgG4 were found in urine samples. Serum/urine ratio of IgG1 and IgG4 correlated well (r=0.73, P<0.0001). Although 5 out of 75 urine samples showed moderately high protein levels (30~55mg/dl) and their anti-B. pahangi IgG4 levels were high, the remaining 70 urine samples were normal (<30mg/dl). These observations suggest that presence of anti-B. pahangi IgG4 in urine, which is utilized for diagnosis, is not due to leak caused by abnormality of kidney function.

C - 02) DIRECT AGGLUTINATION TEST (DAT) WITH URINE SAMPLES FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

MOHAMMAD ZAHIDUL ISLAM¹, MAKOTO ITOH¹, RUSELLA MIRZA², IFTIKHAR AHMED³, XU-GUANG QIU¹, AND EISAKU KIMURA⁴

Department of Parasitology, Aichi Medical University School of Medicine¹, Department of Microbiology, Rajshahi medical College, Bangladesh², Department of Endocrinology and Metabolism, Research Institute of Environmental Medicine, Nagoya University³

Due to high sensitivity, specificity and ease to perform, direct agglutination test (DAT) has been widely accepted for the diagnosis of visceral leishmaniasis (VL) especially in the field. However this method requires blood, which hinders the sample collection in the field particularly with pediatric subjects. In this study we applied urine, as a sample for DAT instead of blood.

We prepared trypsin treated, formalin fixed, and Coomassie brilliant blue stained Leishmania donovani promastigotes as DAT antigen. The quality of the antigen was standardized with the commercially available antigen produced by Koninklijk Instituut voor de Tropen, Amsterdam. Fifty microliter of urine sample was applied without concentration to a well of a V-shaped 96-well microtiter plate, to which 50 µl DAT antigen was added. Then the plate was incubated overnight at room temperature. The results were judged with standard positive and negative samples.

The sensitivity of the assay was 66.7% (38 positives/57 VL samples). The specificity of the assay, determined with 137 non-VL samples, including 59 non-endemic
healthy controls, 58 malaria, 13 tuberculosis and 7 other diseases, was 94.1% (129 negatives/137 non-VL samples).

We previously reported a urine-based ELISA for the diagnosis of VL, which showed 95.0% (57 positives/60 VL samples) sensitivity and 95.3% (204 negatives/214 non-VL samples) specificity. One parasitologically confirmed sample became negative with urine-based ELISA, whereas in urine-based DAT that sample became positive. As the sensitivity of the urine-based DAT is still inferior, we are trying to improve it. Urine-based DAT has several advantages over conventional DAT. (1) Urine collection is non-invasive. (2) The assay system can process more number of samples in one plate, which will reduce the amount of antigen, the cost, time and labour/test.

C - 03) DETECTION OF PLASMODIUM BERGHEI AND P. GALLINACEUM BY ICT P.F./P.V. IMMUNOCHROMATOGRAPHIC TEST

MEIJI ARAI, HIROYUKI MATSUOKA AND AKIRA ISHI

Department of Medical Zoology, Jichi Medical School

ICT P.f./P.v. is an immunochromatographic dipstick test which detects histidine-rich protein, HRP-2 of Plasmodium falciparum. ICT P.f./P.v. can also detect P. vivax and other non-P. falciparum parasites with a pan-specific antibody. Before employing ICT P.f./P.v. for routine screening, extensive evaluation of its usefulness must be made. It is very important to study at which extent of parasitaemia a newly-infected patient shows a positive result by ICT P.f./P.v., and how its reactivity are affected during clinical course. Several groups have already reported such information. However, under field conditions, difficulty in confirming ‘newly-infected’ among the malaria-positive subjects, persistent post-treatment antigenaemia, cross reactivity with rheumatoid factor, and poor skills of local microscopists, should be taken into account. Recently, we found the pan-malarial antibody of ICT P.f./P.v. recognises non-human parasites, P. berghei and P. gallinaceum. In this report P. berghei-infected mice and P. gallinaceum-infected chickens which had been infected by either merozoite injection or mosquito bite, were tested for microscopic examination and ICT P.f./P.v. test. Lowest numbers of parasite in the blood containing P. berghei or P. gallinaceum detected by ICT P.f./P.v. were 8,400 and 10,000 parasites, respectively. Reportedly the pan-specific antibody shows good detection sensitivity on blood samples containing parasites more than 500/µl (7,500 parasites in 15 µl), which is close to our result on P. berghei. Thus the mouse model seems to be useful for the study of detection sensitivity on onset parasitaemia after mosquito bite. Further study on persistent post-treatment antigenaemia is underway. P. berghei-mouse system may provide a good model for evaluation of ICT P.f./P.v. and other rapid diagnostic tests for malaria.

C - 04) ANTI-SERA ANTIBODY PRODUCTION AS A PARAMETER OF ANTI-MALARIA IMMUNITY IN INDIVIDUALS IN THE SOLOMON ISLANDS

MARIKO HATO¹, JUN FU¹, TOSHIHIRO HORI², KAZUYUKI TANABE³, HIROSHI OHMAE⁴, AKIRA ISHII⁵, MASATO KAWABATA⁶, JUDSON L. LEAFASIA⁷ AND NOBUO OHTA¹


Serine-rich antigen (SERA) of Plasmodium falciparum is one of the most promising vaccine candidates against erythrocytic stage of the parasite in humans. Based on the recent in vitro observations showing effective SERA-mediated protective immunity, the present study was conducted to uncover the SERA-mediated protective immunity in individuals in endemic areas of falciparum malaria through the sero-epidemiological approach. People in Guadalcanal, where transmission of falciparum malaria is meso or hyper endemic, were divided into three categories;
slide(-)/symptom(-), slide(+)/symptom(-), and slide(+)/
symptom(+). Number of each group was 70, 19 and 26,
respectively. The N-terminal portion of SERA (SE36’) and
the N-terminal 6 blocks of MSP1 (M1/6) were used for
ELISA. We observed that total IgG production specific for
both SE36’ and M1/6 was significantly higher in slide(-)/
symptom(-) group than in slide(+)/symptom(-) group (p<
0.01). The difference was much more apparent in SERA-
specific IgG levels than that in M1/6-specific IgG. When
we compare IgGs levels in the two groups, no symptomatic
individuals of 15 yr or more showed detectable level of SE
36’-specific IgG3. On the other hand, we did not observe
any difference in polio virus-specific antibodies between the
two groups. Out of 70 slide(-)/symptom(-) group, 14 were
negative for SERA-specific IgG, while all of the group
showed positive IgG to M1/6. Strong association was ob-
served for degree of parasitemia and SE36’-specific IgG.
No individuals with parasite density of 100 and more had
detectable level of SE36’-specific IgG. Together with those,
we concluded that SERA is a promising vaccine candidade
for human falciparum malaria in our seroepidemiological
study in the Solomon Islands.

C - 05) SERODIAGNOSIS OF TUBERCULOSIS USING MPB64 DERIVED FROM MYCOBACTERIUM BOVIS

ATSUKO YOSHIDOME1, SEIJII KANDA1, ITOKO HAYASHI1, MASAO UEMOTO2,
KOICHI TOMODA1, TOSHIKASA NISHIYAMA1

1Dept. Public Health, Kansai Med. University, 2Tsuda Hospital, 3Saiseikai Suta Hospital

It is known that the number of patients for tuberculosis
is gradually increased in the worldwide, including in Japan.
Especially in the developing countries, it’s important to
identify the patients for active tuberculosis. In the previous
study, the MPB64 antigen was efficient by the patch test for
the diagnosis of active tuberculosis. In addition, there was
another report for MPB64 in which PCR analysis was use-
ful to detect the diagnosis of tuberculous pleuritis and men-
ingitis. In this study, we try to find MPB64 has the high
specificity and sensitivity for the diagnosis of the active tu-
berculosis. mpb64 cDNA was kindly donated from Dr.
Matsuo (Nagasaki University) and cloned into the expres-
sion vector by the GATEWAY technology system (invitro-
gen Inc..). The recombinant protein MPB64 was expressed
as a fusion protein with His-tag for the C-terminus and the
M. W. was guessed about 30 kDa. Western blot analysis
was done using a patient’s sera for active tuberculosis and
healthy subjects as a control. The result was shown that a
specific band for 30 kDa was detected with the patient but
not for the control. Therefore this band was also detected
by the immunoreactive with anti-His Tag antibody. So this
was MPB64-specific reactivity for the patient’s serum.

Then MPB64 protein was affinity-purified using His-tag
and the IgG titers in the sera were measured by ELISA
method. The mean value of patients was higher than that of
healthy subject. And, there is a tendency to decrease the
IgG titers time-dependently. It is considered that IgG titers
specific for MPB64 were decreased by the effect of the
treatment progress in sera. These results suggested that
MPB64 might to be a valid antigen for the patient of active
tuberculosis and there is a possibility to use the screening
method of the patient for the active tuberculosis in the field
of developing country.
A METHOD OF BLOOD EXAMINATIONS IN OVERSEAS MOBILE CLINIC
ITS CLINICAL APPLICATION TO THE REMOTE AREAS IN LAO PDR

TAKAAKI ISHIDA, TOSHIMASA NISHIYAMA, HIROYUKI AMANO, YASUKO TAMADA, BOUTHONG SISOUNTHORN, AMPHAVANH PANHANOUVONG, MITSUE UEDA, CHIEKO MIYAKE

Department of Public Health, Kansai Medical University
Curative Department, Ministry of Health, Lao PDR
Aichi Medical Foundation of Diagnostic Technology
Department of Public Health, Aichi Medical University

In this study, we carried out overseas mobile clinic in Lao PDR from April to May (for 21 days) in 2002, and examined the serum biochemical tests and blood examinations using newly developed filter paper plasma skimming film which is easy to separate without centrifuge.

The sera were collected from the inhabitants of DakEuy village (168 subjects) at SeKong Province and NonTao village (68 subjects) at OudomXay Province. In this time, we were able to examine by the skimming filter for GOT (AST), GPT (ALT), γ-GTP, BUN, Creatinine, Total cholesterol, HDL-cholesterol, Triglyceride, Amylase and Uric acid by the filter paper for Hemoglobin, HBs Ag and HCV Ab by the rapid blood sugar analyzer for Blood Sugar.

The results of these examinations were GOT 35.5 ± 16.7 IU/l, GPT 21.3 ± 5.7 IU/l, γ-GTP 35.5 ± 14.1 IU/l, BUN 11.5 ± 3.4 mg/dl, Cr. 0.4 ± 0.1 mg/dl, T CHO 135.0 ± 21.2 mg/dl, HDL-CHO 31.7 ± 6.4 mg/dl, TG 107.9 ± 45.6 mg/dl, AMY 113.4 ± 82.0 U/l, UA 3.3 ± 0.9 mg/dl, Hb 16.1 ± 1.8 g/dl, BS 88.6 ± 13.6 mg/dl, in DakEuy village. In NonTao village, GOT 20.1 ± 12.1, GPT 21.3 ± 5.7, γ-GTP 33.9 ± 15.4, UA 5.4 ± 1.0, Hb 13.3 ± 2.1, BS 94.8 ± 18.3. In the 4 cases of liver disfunction, we have a chance to measure HBs Ag and HCV Ab. Two cases were positive for HCV Ab, but no case was positive for HBs Ag.

There was no person who showed higher than upper range of normal limit of Total cholesterol, Uric acid and renal function. And, the patient of diabetes mellitus was not diagnosed in these inhabitants. In the DakEuy village, hemoglobin values of the inhabitants were higher, because it might be influence that this village located in the highland over 1,000m altitude.

Therefore, it was concluded that these methods (plasma skimming film and filter paper), which were developed as screening test in Japan, were suitable for the mobile clinic of rural areas in the developing country.

COMPARISON OF ULTRASONOGRAPHIC IMAGES BETWEEN SCHISTOSOMA JAPONICUM AND S. MEKONGI INFECTIONS

HIROSHI OHMAE, YUICHI CHIGUSA, HIDEHIRO OTAKE, ORLAMDO S. SY, EUINCE ILAGAN, MUTH SUNUON, DUONG SOCHET, HAJIME MATSUDA

In order to estimate liver fibrosis due to Schistosoma japonicum and S. mekongi infections, ultrasonographic (US) examination were used in the schistosomiasis endemic areas of Philippines and Cambodia. Typical liver damage due to schistosomiasis japonica can be detected by US, as “network pattern” or “fish scale pattern”. Advanced bridging fibrosis reveals these patterns, and periportal fibrosis shows other changes such as thickening of portal vein wall. Severe thickening of portal vein wall and collateral vessels are frequently found in elder cases with network pattern.

But in young cases, we find no relationship between network pattern and periportal fibrosis detected as thickening of portal vein wall.

In S. mansoni infections, based on the degree of periportal fibrosis, liver fibrotic changes are classified into some patterns and these patterns do not include network pattern. In S. mekongi infections, typical network pattern has not be found. And except for the pattern like irregular fatty liver,
most of the patterns of liver fibrosis are similar to those of schistosomiasis mansoni. US examination is broadly used in developing countries as one of the appropriate techniques, because this technique is easy and cheap. US has been used for morbidity studies of schistosomiasis. In *S. mansoni* infections, for morbidity studies, we have a standard criteria of US diagnosis. In *S. japonicum* and *S. mekongi* infections, standard criteria of US diagnosis is expected.

**C - 08) COMPARISON OF ULTRASONOGRAPHIC IMAGES OF LIVER AND SPLEEN BY AGE GROUP AND THE RELATIONSHIP BETWEEN FREQUENCY OF PRAZIQUANTEL TREATMENT AND ULTRASONOGRAPHIC IMAGES OF PATIENTS SUFFERING FROM SCHISTOSOMA JAPONICUM AND S. MEKONGI INFECTION IN THE PHILIPPINES AND IN CAMBODIA**

YUICHI CHIGUSA¹, HIROSHI OHMAE², EUNICE J. ILAGAN³, NINA G. BARZAGA⁴, MUTH SINUON⁵, CHEAM SAEM⁶, DUONG SOCHEAT⁷, MASATO KAWABATA⁸ AND HAJIME MATSUDA⁹

Department of Tropical Medicine and Parasitology, Dokkyo University School of Medicine¹, Basic Medical Sciences, University of Tsukuba², Schistosomiasis Control Team, Department of Health, Philippines³, University of the Philippines⁴, National Malaria Center⁵, Provincial Health Department, Kratie⁶, Ministry of Health, Cambodia, International Center for Medical Research, Kobe University⁷

We conducted studies in Oriental Mindoro, Philippines for schistosomiasis japonica (Sj) during July and August 2002 and in Kratie, Cambodia for schistosomiasis mekongi (Smek) during May and June 2002. The patients were examined by ultrasonography (US) and questioned concerning the frequency of praziquantel (PZQ) treatment since 1995, in which the drug were given at a dosage of 40mg/kg body weight in Cambodia and 40-50mg/kg body weight in the Philippines. The number of patients examined were 234 (170 males: 64 females) in the Philippines and 382 (214 males: 168 females) in Cambodia.

The patients were classified into two groups, *i.e.* 19 years of age or younger and those at least 20 years old. The frequency of PZQ treatment since 1995 was classified as A) 0 to 2 times, B) 3 to 5 times and C) more than 6 times. Spleen size was estimated by both US and palpation and was classified as A) within normal limits (WNL), B) less than 15 cm from the costal arch (less than 15) and C) over 15 cm from the costal arch (over 15). Periportal thickening of portal vein and collateral circulation of the splenic vein were also observed and recorded by US.

Percentages of patients with splenomegaly in groups A) WNL, B) less than 15 and C) over 15 were 96.0%, 4.0% and 0% in the Philippines and 80.2%, 19.8% and 0% in Cambodia, respectively among the younger patients, and 91.8%, 8.2% and 0% in the Philippines and 35.3%, 57.4% and 7.4% in Cambodia, respectively among the older patients. Periportal thickening of the portal vein was observed in 54.0% (Sj) and 50.5% (Smek) of younger patients and 53.3% (Sj) and 66.3% (Smek) of those over 20 years of age. Collateral circulation of the splenic vein were observed by US in 0% (Sj) and 4.2% (Smek), respectively in patients of the younger age group but in 2.2% (Sj) and 30.5% (Smek), respectively of the older age group.

As shown in the results, frequent PZQ treatment reduced the incidence of periportal thickening of the portal vein but not those of hard enlarged spleen and collateral circulation of the splenic vein. These observations indicate that early diagnosis and PZQ treatment of schistosomiasis japonica and mekongi is important.
THE INFLUENCE OF SCHISTOSOMA HAEMATOBIUM INFECTION ON VOIDING FUNCTION

KANJI WATANABE¹, MITSURU NOGUCHI², KOICHIRO NOMATA³, NGETHI D MUHOHO.⁴ AND YOSHIKI AOKI⁵

¹Department of Parasitology, Institute of Tropical Medicine, ²Department of Urology, School of Medicine, Nagasaki University, ³Kenya Medical Research. Institute, Kenya

To estimate the influence of the Schistosoma haematobium infection on the voiding function quantitatively, urinary flow velocity and residual urine volume after urination in primary school boys were measured using portable uroflow meter (RMS-100, Tyouryou-systems, Japan) and ultrasound machine (Bladder Scan BV3000, Alcare, US.).

This study was done in two areas in Kenya during February, 2002. One area was Kiambu district near Nairobi, the capital of Kenya. Schistosoma haematobium infection was not endemic in this area. The other study area was Kwale district, Coast Province, which lay in the east of the Kenya. Schistosoma haematobium infection was endemic in this area. Study subjects were mainly primary school boys because the uroflow meter was designed to measure the male voiding function only. Before examination, 500 ml of juice was given to each students. When they felt bladder full, they started to pass the urine into the uroflow meter. The uroflow meter measured the maximum flow velocity, average flow velocity, total voiding time, total voiding volume. After urination, the students were examined about residual urine volumes. The S. haematobium eggs number in 10 ml of urine was also examined using Nucleo-pore filer.

The eggs count revealed that the boys in Kiambu were not infected with S. haematobium but 50% of the boys in Kwale were infected. The uroflow meter analysis showed no difference in the proportion of the maximum uroflow velocity to the total voiding volume between boys in Kiambu and in Kwale. Furthermore the residual urine volumes were also not different between the boys. The same kind of examination to the adults showed that the proportion of the maximum uroflow velocity to the total voiding volume in Kwale adult males was lower than that in Kiambu adult males. From these results, we concluded that S. haematobium infection did not cause the voiding dysfunction in the children but it caused the dysfunction in adults.

EPIDEMIOLOGICAL SURVEILLANCE ON SCHISTOSOMIASIS IN MOUNTAINOUS REGIONS IN SICHUAN

CHENG-KUO CHUANG¹, NORIAKI KAJIHARA², HONG-ZHI YIN³, LIAN-GUO ZHAO³, KAI-YOU SHA³, MASARU MINAI AND YASUO NAKAJIMA³

Department of Parasitology and Immunology, Faculty of Medicine, University of Yamanashi¹, Yamanashi Prefectural Institute for Public Health² and Anti-schistosomiasis Station of Xichang City, Sichuan, China³

The area infested and the population infected with Schistosoma japonicum in China, reduced after the control program. The infested area and the infection rate, however, appear to have increased in some highlands in Sichuan and Yunnan. The primary objective was to study the epidemiology and the factors impeding the control in the areas.

Surveyed were five villages near Qionghai Lake, Xichang City, Sichuan Province, in which the fecal examination of villagers in 2000 had revealed the positive rate for S. japonicum of over 30%, of around 10% or of 3-5%; Two sections from each village were investigated.

Results: Cercaria-positive Oncomelania snails were found in a section of Xinmin Village (7.7%); that of Zhan-glin Village (0.9%) and that of Gucheng Village (0.7%). The trapped and dissected field mice were free of schistosomes.

In Xinmin Village where the egg-positive rate of over 30% had been found in the previous examination, the fecal samples from the villagers one year after the treatment revealed the positive rate of 33.9% in Sections 3 and that of 65.6% in Section 7 for miracidia.

Discussion: The villagers cultivate vegetables using human excreta as manure and working bare-footed. They wash in irrigation ditches. The snail inhabits in the irrigation canal near the hamlet and at the bottom and side walls of the irrigation ditches in the farms. More positive snails were found near the houses. The dominant species of field mice is Apodemus chevrei, which seems to supply ova
poorly in spite of its frequent contact with water. Although
the infected human and domestic animals were treated, the
infection rate has not reduced in a village. The sanitary life-
style has to be changed and the household water has to be
independent of the farming water to solve the problem.

C - 11) PARASITOLOGICAL SURVEY AND RELATED HEALTH AWARENESS INVESTIGATION
BY QUESTIONNAIRE AT A VILLAGE IN THE CENTRAL REGION IN GHANA

YOSHIO OSADA1,2, KWABENA M. BOSOMPEM2, SOMEI KOJIMA3, NOBUO OHTA4

Department of Parasitology and Tropical Public Health, University of Occupational and Environmental Health1,
Noguchi Memorial Institute for Medical Research/JICA infectious Diseases Project2,
The Asian Centre for International Parasite Control3,
Nagoya-city University, Graduate school of Medical Sciences, Department of Molecular Parasitology4

Background: In Ghana, as well as in other sub-Saharan
countries, many parasitic infections affect people. In such
situation, irrigation projects have been introduced to in-
crease agricultural productivity and income. It has been
also pointed out that irrigation often results in expansion of
mosquito-borne and/or water-borne parasitic infections.
Therefore, continuous efforts to keep health of residents
living around the area are important. We conducted health
education on endemic parasitic diseases in a village (Oky-
ereko, located in the coastal area in Ghana) in which a small-
scale irrigated agriculture promotion project (SSIAPP) by
JICA was under implementation. Here we show the results
of a baseline survey of endemic parasitic diseases and
health awareness investigation in the village. Methods:
Urine and stools have been collected from residents in the
district for parasitological assay. Eggs in the stools were
detected by Kato-Katz method and eggs in the urine were
detected by Nucleopore membrane filtration. Question-
naires on awareness of parasitic diseases present in the vil-
lage were administered to residents aged more than or equal
to 10 years old. Results and Discussion: Schistosoma hae-
matobium infection was the most prevalent disease among
those we examined. Approximately 50% of the residents
aged 10 to 29 years old were infected. Second highly
prevalent helminth infection was hookworm infection,
which affects 23% of the residents. Schistosoma mansoni,
Trichuris trichiura and Ascaris lumbricoides infections
were also observed. Additionally, in a previous study, lymph-
atic filariasis (Wuchereria bancrofti infection) was shown
to be endemic in this village (Dzodzomenyo, et al., 1999).
Thus, as for helminthiasis, we can conclude that urinary
schistosomiasis, hookworm infection and lymphatic filaria-
sis are highly endemic in this village. Analysis of aware-
ness and behavior of residents on such parasitic diseases-
related symptoms revealed that they had a tendency to seek
medicines from chemists or peddlers rather than to see doc-
tors. Only approximately 20% and 10% of the persons who
had disease-related symptoms tend to see doctors, in the
case of malaria and urinary schistosomiasis, respectively.
Analysis of their knowledge on transmission route of para-
sitic diseases showed that they had a relatively correct
knowledge of transmission of malaria, urinary schistosomi-
asis and intestinal infections. In contrast, they did not know
the relationship of mosquito bite and lymphatic filariasis.
This fact may be due to the long time progression of the
chronic symptoms of the disease.
C - 12) DAMAGE IN CERCARiae OF SCHISTOSOMA MANSOnI INDUCED BY PRESSURE JAR IN WATER

NOBU. OHWATARI¹, YASUNORI. FUJIMAKI², ATSUSHI. KUMATORI³, KAN. TORIYAMA⁴, EIKO. KANEDA⁵

Department of Environmental physiology¹, Parasitology², Biochemistry³, Pathology⁴, Social environment⁵, Institute of Tropical Medicine, Nagasaki University

We experimented about influence of the water pressure vibration for a cercariae of Schistosoma mansoni. Cercaria comes out of a shell of intermediator into water by light, and cercaria has a form peculiar which connection department of head and tail is narrow. Therefore, mechanical energy of the water pressure vibration may be influenced to cercaria with strong damage.

In the first, we were examined some following load to cercaria of each group with 50 cercaria in water. The ratios of tail loss were 10.0% by magnetic rotation field for 30 minute, 13.9% by static water pressure for 30 minute, 27.0% by magnetic rotation field and low speed stream for 30 minute, 87.2% high-speed stream for 10 minute, respectively. Then control group was 0% at ratio of tail loss.

In the next, cercaria were exposed mechanical energy by 46 kHz of ultrasonic wave generator was load for 1 minute, 2 minutes, 3 minutes and 5 minutes in water. The connection department of head and tail was amputated by the mechanical energy, and the ratio of tail loss reached 100% for less than 4 minutes as a result of regression line in statistical analysis.

Furthermore, we were examined the ultrasonic wave load to infected shell of intermediate host for one minute. Body of the shell took shelter in the depths of conch during ultrasonic wave exposure. Cercaria without tail was 99.2% in which cercaria flowed out of the shell after ultrasonic wave exposure only one minute.

In this study, it was suggested that following. The tail of cercaria which appeared from a shell evolved for a purpose to arrive at final host in a short time. But the small part between head and tail of cercariae was weak against high-speed stream or water pressure vibration induced by ultrasonic wave generator. Interference pattern reflected of ultrasonic wave at inside wall of a shell was very effective for amputation of cercariae, in which configuration of the conch that the depths narrow progressively. Therefore, it is most effective to expose shell to pressure jar in water for amputation of cercariae tail.

C - 13) COMPARISON OF INTESTINAL PARASITIC INFECTIONS AND MALNUTRITION IN SCHOOLCHILDREN IN URBAN AND RURAL AREAS IN HANOI, VIETNAM

YASUNORI FUJIMAKI¹, DAO TO QUyen², YUKI SATO³, NGUYEN THI DANG⁴, HA HUY KHO¹, DO THI KIM LIEN⁵, YOSHIKI AOKI¹, SHIGERU YAMAMOTO³, FUSAO OTA⁵

¹Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, ²National Institute of Nutrition, Hanoi, Vietnam, ³Department of Applied Nutrition, ⁴Department of Food Microbiology, School of Nutrition, The University of Tokushima, ⁵National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam

Surveys on parasitic infections and nutritious status in schoolchildren were conducted in urban(U) and rural(R) areas of Hanoi, Vietnam. Data of both parameters obtained from 75 and 104 children in U and R respectively were analysed and compared for the prevalence and intensity of parasitic infections and nutritious status between the two areas. The school children were examined for parasites in their fecal samples, their daily food intake, weight and height, and anthropometric indices following the Kato-Katz technique, '24 hour recall' method and the standard US National Center for Health Statistics charts and tables, respectively. Results: 1) Overall prevalence of parasitic infections was significantly higher in R (96.2%) than that in U (83.0%). In both areas Trichuris trichiura was the most dominant parasite. 2) The geometric mean of EPG (egg counts per gram feces) of Ascaris and Trichuris was significantly higher in R than that in U. 3) Weight and height were significantly greater in U than those in R. 4) Weight-for-age analysis showed that 13 (18.1%) children in U and 59 (56.7%) in R were malnourished. 5) Height-for-age analysis showed that 16 (21.3%) children in U and 51 (49.0%) in R were malnourished. 6) A stratified analysis by Mantel-Haenszel chi-square test showed no relationship between the prevalence of infections, malnutrition and the
sites examined. 7) No significant differences in total calorie intake was observed between the normal and malnutritional groups. **Conclusions:** Schoolchildren in the rural area in Hanoi, Vietnam had a higher prevalence and intensity of parasitic infections, and a higher rate of malnutrition than those in the urban area. There was no significant difference between nutrition status and the prevalence of parasitic infections with the same calorie intake in normal and malnutritional groups. (*Supported partly by Grants-in-Aid (No. 11691211) from the Ministry. Educ. Sci. Sport. & Cult, Japan).

**C - 14) DETECTION OF MICROORGANISMS IN TAP WATER IN INDONESIA AND THAILAND**

SHOJI UGA¹, TAKUYA ODA², KENJI KIMURA³, DAI SUKE KIMURA⁴, K OESDARTO⁵, MARGONO⁶, KUNAU KU⁷ AND NUALTA⁸

Department of Medical Technology, Faculty of Health Science, Kobe University School of Medicine⁹, Water Quality Laboratory of Kobe City Waterworks Bureau¹⁰, Water Quality Center, Maezawa Industry Inc.¹¹, Airlangga University, Indonesia¹², University of Indonesia¹³, Prince of Songkla University, Thailand¹⁴

Water samples were directly collected from house faucets at each collection site and filtered through membranes. The membranes were brought to Japan and examined for protozoan parasites by immunomagnetic separation. Coliform and *Escherichia coli* were examined at each sample collection site using commercially available kits. A total 115 water samples were examined and 37 (32%) were revealed positive for four types of microorganisms. These microorganisms were two kinds of bacteria (coliform and *Escherichia coli*) and two species of protozoa (*Giardia intestinalis* and *Cryptosporidium parvum*). Of those detected, coliform was the most common and was found in all three areas with a mean detection rate of 30%. *G. intestinalis* and *C. parvum* were found at the rates of 9% and 1%, respectively. Bacterial contamination was closely related to the concentration of residual chlorine but not protozoan contamination. The water samples that were positive for any of the four microorganisms showed a tendency to have lower residual chlorine concentrations and higher turbidities compared with negative samples. It is important to supply safe water in order to maintain people’s health because most of the people surveyed ordinarily drank tap water without treating it. Continued efforts are needed to maintain and improve drinking water quality.

**C - 15) EPIDEMIOLOGICAL ANALYSIS OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* INFECTION IN INDONESIA**

YOSHIO ICHINOSÉ¹, TAKAO TSUIJ⁵, EDI KAWASE⁶, TEIZO TSUKAMOTO⁶, YASUHIKO WATAYA⁶, TAKUJI KUMAGAI⁷, MASAAKI SHIMADA², YOES P. D.², EDDY B. WASITO⁸ AND MASAAKI IWANAGA⁷

Department of Bacteriology¹ and Research Center for Tropical Infectious Diseases², Institute of Tropical Medicine, Nagasaki University, Department of Microbiology³, School of Medicine Fujita Health University, Osaka Prefecture Institute of Public Health⁴, Wataya Clinic⁵, Kumagai Clinic⁶, TDRC, Airlangga University⁷ and Department of Bacteriology, School of Medicine, Ryukyu⁸

Enterohemorrhagic *Escherichia coli* (EHEC), which can occasionally cause big outbreaks and sporadic cases in developed countries, is rarely isolated as causative agents of diarrheal diseases in developing countries. In this study, we perform an etiological investigation on diarrheal cases and a carrier rate of enteropathogens among non-diarrheal cases from two medical facilities in Surabaya, focusing on EHEC, and the carrier rate of EHEC among domestic animals such as cattle, pigs and goats. In addition, serological analysis on the serum IgG level of anti-VT1 and anti-O157 LPS antibodies among Indonesian and Japanese pediatric cases was performed to clarify real situation of EHEC infection in Indonesia.

It was revealed that six strains of *Vibrio cholerae* O1,
six strains of Shigella, three stains of Salmonella, one strain of enteroinvasive Escherichia coli, 19 strains of enteropathogenic Escherichia coli and only one strain of EHEC strain (O8:H4) were isolated from diarrheal and non-diarrheal patients. Thirteen strains of EHEC were isolated but no EHEC O157 was isolated from cattle stool samples. Three strains of EHEC O157 were isolated from goats. No EHEC was isolated from pigs. The production pattern of verotoxin was completely identical to that of toxin genes detected by PCR. The fingerprinting pattern of genomic DNA by PFGE was almost identical to those of strains isolated from sporadic cases in Nagasaki prefecture.

The serum levels of anti-VT1 and anti-O157 antibody determined by ELISA among pediatric cases in Indonesia increase by age since neonatal period, while those in Japan seem to decrease once after birth until they begin to increase at four to five years of age. These findings suggest that previous exposure to EHEC or VT1, or VT1 related antigens could evoke and sustain high levels of serum antibody against VT1 and O157 LPS.

C - 16) THE PROBLEM ON MALARIA TREATMENT
ABOUT THE DELAY OF DIAGNOSIS AND TREATMENT

ETSUSHI OKUMURA1, TAKASHI NISHIMORI2, KEISUKE HASHIMOTO3, KENICHI KATSU4, TSUNEJI ARAKI5

Department of Medical Technology, Kochi Gakuen Junior College1
Department of Clinical Technology, Kubokawa Hospital2
Department of Internal Medicine, Osaka Police Hospital3
Department of Internal Medicine, Osaka Medical College4
Department of Internal Medicine, Shinsei Hospital5

In our experience to treatment for five cases of malaria patients, we had great deal of serious situation. That was lateness to the diagnosis and treatment.

Case 1: A middle school boy of 15 years old caused by ovale-malaria; His diagnosis was two days behind the admission and treatment was five days lingered because of difficulties to obtain anti-malaria medicine.

Case 2: A trading company employee of 44 years old caused by falciparm-malaria; He made a long stay in Lagos in Nigeria from 1973 to 1975 and had arisen a complication of cerebral symptoms, but Chloroquine and Primaquine were very good efficacious to him nevertheless.

Case 3: A woman of 40 years old university lecturer in English caused by vivax-malaria; She also started to apply the medicine after six days of the diagnosis. The reason was one and the same as case 1.

Case 4: A Vietnamese seaman of 33 years old caused by vivax-malaria; He had not been confirmed his recovery perfectly. Because his cargo boat had left Osaka port to Pusan in Korea just after the diagnosis on this evening.

Case 5: A male patient, 41 years old American, who teaches English at the Berlitz school in Osaka; He had recovered before becoming encephalopathy, although he was a case of Chloroquine-resistant falciparum-malaria. He had taken instant effect on Quinine-injection in spite of renal disorder.

Fortunately, we had not experienced miserable cases.
To countermeasure the delay of diagnosis
1. Precise sampling and exact observation (Giemsa stain; Buffer solution, not pH6.4 but pH7.4).
2. Enlightenment of Polymerase chain reaction technique, Fluorescent antibody technique etc..

To countermeasure the delay of treatment
1. Vid. Today’s Therapy. Vol. 44. 2002 (Edit. Sachio Takasu M.D. Igaku-shoin Co. Ltd). In this textbook, the name of Organization in Japan which keep anti-malaria medicine is printed with telephone number.
2. Qinghaus; ask Guilin No2 Pharmaceutical Factory Guangxi, China Republic.
C - 17) A SEVERE CASE OF *PLASMODIUM FALCIPARUM* INFECTION COMPLICATED WITH BLACK WATER FEVER.

KOYAMA, K.

C - 18) CORRELATION BETWEEN THE RECOGNITION BY OBSTETRICIANS ABOUT CONGENITAL TOXOPLASMOSIS AND THEIR CLINICAL ACTIVITIES

KOJI NAOI, OSAMU HAYAKAWA, YOSHIO SAKANO AND AKIHKO YANO

Department of Infection and Host Defense, Chiba University Graduate School of Medicine
Obihiro-Tokachi Association of Obstetricians and Gynecologists

In the present study, correlation between the recognition held by obstetricians about congenital toxoplasmosis (CT) and their clinical activities was investigated with a questionnaire for 15 subjects, or obstetricians in Tokachi-Obihiro area, Hokkaido, Japan. Besides their attributes, obstetricians were asked about their clinical activities on CT, such as serum antibody tests and explanation for pregnant women, as well as their recognition about the risk of CT. According to the present study, obstetricians who are negative for the explanation are relatively older with longer clinical experience. Obstetricians who have experienced some cases of CT are more positive for the explanation than those who have not. For serum antibody test to *T. gondii*, on the other hand, no correlation is observed with statistical significance. On the recognition about the risk of CT, self-employed obstetricians have lower estimates on the risk of infection for pregnant women (RIW) as well as that for fetus (RIF) than employed obstetricians. Obstetricians who have not experienced any cases of CT also hold lower estimates on RIW and RIF than those who have.

Obstetricians for medical clinics indicate their lower estimates on the damage to fetus (DF) than those for private general hospitals. It is also indicated, the older and with the longer clinical experience, the lower estimates on RIW, RIF and DF. The number of cases of CT, experienced by obstetricians, positively correlates with their estimates on RIF.

The present study implies that relatively older self-employed obstetricians for medical clinic, with longer clinical experience but few cases of CT, are typically negative for the explanation, backed by their lower estimates on the risk of CT. The results of the present study, we believe, reflect a tragedy that CT has been paid less attention here in Japan. As we showed before, the lower risk estimates on CT are not in line with recent epidemiological evidence. Comprehensive epidemiological research is required to inspect their recognition from the view point of EBM (Evidence Based Medicine).
Original article
Sato, Y., Iwagami, M., Iwashita, J., Abe, T., Yukawa, M., Blas, B. L., Kawashima, K., and Agatsuma, T.
Phylogenetic status of a lung fluke in the Philippines based on mitochondrial genome

Phylogenetic relationships of snails of the genera Oncomelania and Tricula inferred from the mitochondrial 12S rRNA gene

Proceedings of the 43rd Annual Meeting of Japanese Society of Tropical Medicine

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内容

誌

原著

ミトコンドリアゲノムに基づく、フィリピン産肺吸虫の系統学的解析

若林、石上、秋敏、若下、渋、阿部、連也、

種間、Bayani L. Blas、川崎健治郎、西美、

ミトコンドリアゲノム解析から推定した、Oncomelania 属及び

Tritous 属の系統関係

岡本、政裕、Chen-Tson Lu、Wilfred U. Tiu、Dongshuan Qiu、Pranadi Hadidjaja、

Suchart Upatham、杉山、広、田口、角弘、貞久、齊藤、通秀、

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