V. Acknowledgments

I express my sincere gratitude to Professor Naomi Tanaka of Institute of Clinical Medicine, University of Tsukuba for his guidance and supports of the present study.

During the whole of my graduate school term, Associate Professor Yasushi Matsuzaki of Institute of Clinical Medicine, University of Tsukuba supported me kindly and intensely. I wish here to express my appreciation for his supports.

I have sincere thanks for Dr. Shinich Itoh (Tsukuba Medical Center Hospital, Ibaraki) and Dr. Mikio Doy (Director, Ibaraki Prefectural Institute of Public Health, Ibaraki) for valuable advice and animated discussions throughout the present study.

I am also deeply grateful to Assistant Professor Junich Shoda of Institute of Clinical Medicine, University of Tsukuba for valuable advice.
### Table 2-1

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen index</th>
<th>No. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.3 ± 0.1</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>5.3 ± 0.5</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>12.7 ± 1.0 *</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>11.8 ± 0.9 *</td>
<td>15</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SE.

*P < 0.001 vs. groups 1 and group 2

group 1: normal control mice, group 2: Con A

group 3: GVHR, group 4: GVHR + Con A
**Table 2-2**

Changes of autoantibodies titres

<table>
<thead>
<tr>
<th>Group</th>
<th>AMA</th>
<th>ANA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.123 ± 0.019</td>
<td>0.054 ± 0.010</td>
</tr>
<tr>
<td>2</td>
<td>0.134 ± 0.010</td>
<td>0.056 ± 0.013</td>
</tr>
<tr>
<td>3</td>
<td>0.442 ± 0.045 *</td>
<td>0.840 ± 0.138 *</td>
</tr>
<tr>
<td>4</td>
<td>0.431 ± 0.039 *</td>
<td>0.650 ± 0.093 *</td>
</tr>
</tbody>
</table>

AMA=antimitochondrial antibodies    ANA=antinuclear antibodies
Results are presented as mean ± SE.  *P < 0.001 vs. groups 1 and group 2
group 1: NML, group 2: Con A
group 3: GVHR, group 4: GVHR + Con A

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Table 3-1

Change of spleen index

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen inde:</th>
<th>No. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9 ± 0.4</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>15.7 ± 1.9  *</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>15.4 ± 3.5  *</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>23.5 ± 3.6  * †</td>
<td>8</td>
</tr>
</tbody>
</table>

Mean ± S.D. *P < 0.0001 vs. group 1 †P < 0.01 vs. other groups
There was no significant difference between groups 2 and 3.
group 1: NML, group 2: GVHR
group 3: GVHR+Control mAbs, group 4: GVHR+Anti-IL-10
Figure 2-1

<table>
<thead>
<tr>
<th>Group 1 (NML)</th>
<th>Normal control mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 (Con A)</td>
<td>** ↑  † ↑</td>
</tr>
<tr>
<td>Group 3 (GVHR)</td>
<td>* ↑ † ↑</td>
</tr>
<tr>
<td>Group 4 (GVHR + Con A)</td>
<td>day 0 day 5 day 14</td>
</tr>
<tr>
<td></td>
<td>* Cell transfer   ** Con A  † Sacrifice</td>
</tr>
</tbody>
</table>

Experimental design.
* B6 spleen T cells were injected into F1 (bm12 x B6) mice.
** Con A (Concanavalin A) was injected intravenously at a dose of 15 mg/Kg.
† Sacrifice.
Serum level of transaminase.

(A) AST, (B) ALT. At day 14 (9 days after concanavalon A (Con A) injection) group 4 (graft-versus-host reaction + Con A) and at day 9 group 2 (Con A) revealed the increase of serum transaminase. Results represented as Mean ± SE of each experimental group (n=8). *P<0.05, **P<0.001.
Figure 2-3 legend

Morphological changes in graft-versus-host reaction (GVHR) hepatic lesions by concanavalin A (Con A).
In the liver of group 3 (GVHR), mononuclear cell infiltration was observed in the portal area (A). Group 4 (GVHR + Con A) showed increased cellular infiltration and foci of piecemeal necrosis (B). Intraepithelial mononuclear cells of bile ducts were noticed in group 4 (C) as well as in group 3. In contrast, quite mild cellular infiltration was shown in group 2 (Con A; D). Granulomatous lesions in the portal area (E) and bridging necrosis (F) were also observed.
A to E, H&E staining. F, Silver staining.
Original magnification [A, B, D, E] x 40, C x 200 and F x 25.
Figure 2-4 legend

Relative amounts of cytokine mRNA prepared from liver-infiltrating CD4+ T cells.
The relative amounts of fluorescence intensity from each group were measured from six independent experiments. (A) Interferon-γ / β-actin ratios were 0.27 ± 0.04 in group 1, 0.41 ± 0.04 in group 2, 0.57 ± 0.09 in group 3 and 0.77 ± 0.07 in group 4. (B) Interleukin-10 / β-actin ratios were 0.14 ± 0.01 in group 1, 0.29 ± 0.02 in group 2, 0.59 ± 0.06 in group 3 and 0.51 ± 0.07 in group 4. (Mean ± SE).

*P<0.05, **P<0.001. N.S., not significant.

group 1: NML, group 2: concanavalin A (Con A), group 3: graft-versus-host reaction (GVHR), group 4: GVHR + Con A.
Figure 3-1 legend

Deterioration of GVHR hepatic lesions by anti-IL-10 antibodies. In the liver of group 2 (GVHR), mononuclear cell infiltration was observed in the portal area (A). There was no difference between groups 2 and 3 (GVHR+Control mAbs; B, D) with regard to the extent of the portal cellular infiltration. In contrast, group 4 (GVHR+Anti-IL-10) showed a significantly higher degree of cellular infiltration (C, E). Focal intraepithelial lymphocyte infiltration and the loss of continuity of the bile duct wall were observed in group 4 mice (F). A to E, H&E staining. F, Masson's Trichrome staining. Original magnification [A, B, C] x 13.2, [D, E] x 50, F x 66.
Image analysis of portal cellular infiltration area.
The area (μm²) of infiltrated cells from five portal areas were measured for each specimen by using NIH Image. The mean of the cellular infiltrated portal area was significantly increased in group 4 (GVHR + Anti-IL-10) compared with groups 2 (GVHR) and 3 (GVHR + Control mAbs). The data represent means ± SD. Each group consisted of 8 mice, except for group 3 (3 mice). *P<0.0001 compared with groups 2 and 3.
Figure 3-3

A

The relative IFN-γ mRNA

+ 25
+ 20
+ 15
+ 10
+ 5
0

group 1  group 2  group 3  group 4

B

The relative IL-4 mRNA

+ 16
+ 14
12
10
8
6
4
2

group 1  group 2  group 3  group 4
mRNA expression levels of IFN-γ and IL-4 by real-time PCR.
IFN-γ mRNA (A) and IL-4 mRNA (B) in liver-infiltrating lymphocytes was measured by real-time PCR. Relative quantification was performed using GAPDH as an internal standard. The data represent means ± SD. The IFN-γ expression levels of groups 2 (GVHR) and 3 (GVHR + Control mAbs) were higher than that of group 1 (normal control mice) (*P<0.05), whereas there was no significant difference between groups 2 and 3. Concerning IL-4 mRNA, there was no significant difference among groups 1, 2 and 3. The expression levels of both IFN-γ (†P < 0.001) and IL-4 mRNA (‡P < 0.0001) were increased by neutralizing IL-10 in group 4 compared with other groups. Each group consisted of 6 samples, except for group 3 (3 samples).
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Chapter I


Chapter II


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