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

Table 2-1

Change of spleen index

Group	Spleen index	No. of mice
1	3.3 ± 0.1	12
2	5.3 ± 0.5	8
3	12.7 ± 1.0 *	12
4	11.8 ± 0.9 *	15

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

Group	AMA	ANA
1	0.123 ± 0.019	0.054 ± 0.010
2	0.134 ± 0.010	0.056 ± 0.013
3	0.442 ± 0.045 *	0.840 ± 0.138 *
4	0.431 ± 0.039 *	0.650 ± 0.093 *

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

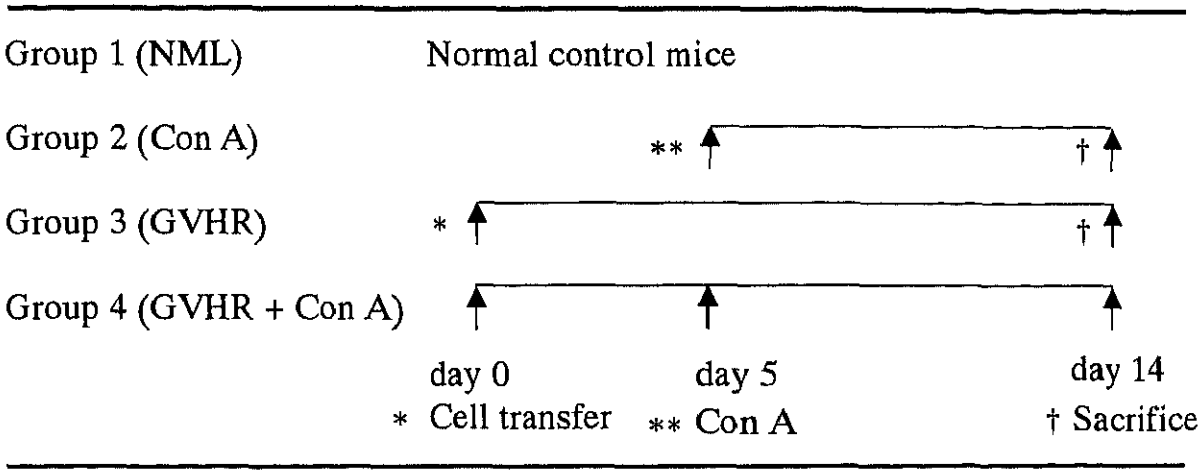
Table 3-1

Change of spleen index

Group	Spleen inde:	No.of mice
1	2.9 ± 0.4	6
2	15.7 ± 1.9 *	8
3	15.4 ± 3.5 *	3
4	23.5 ± 3.6 * †	8

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



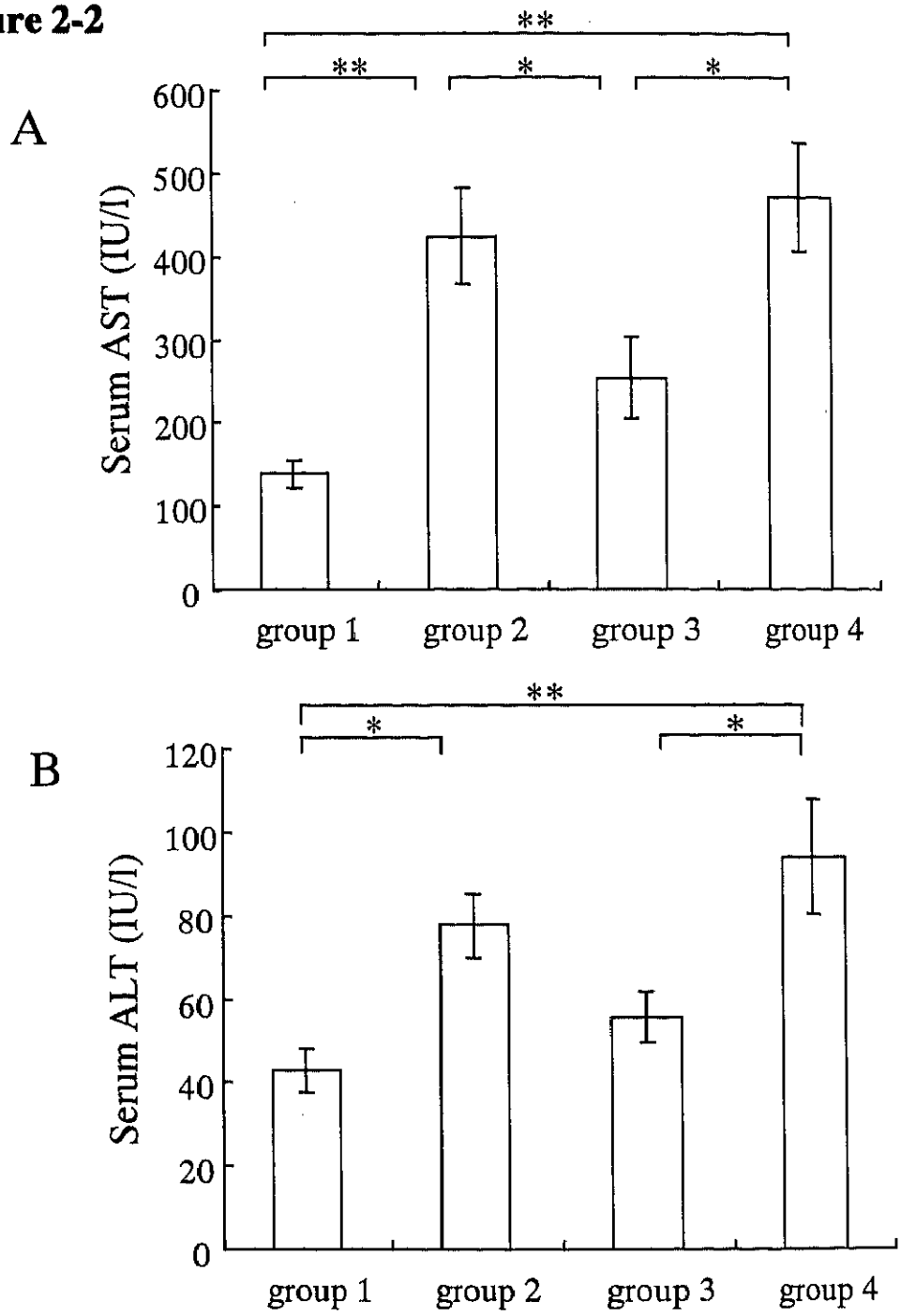
Experimental design.

* 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.

Figure 2-2

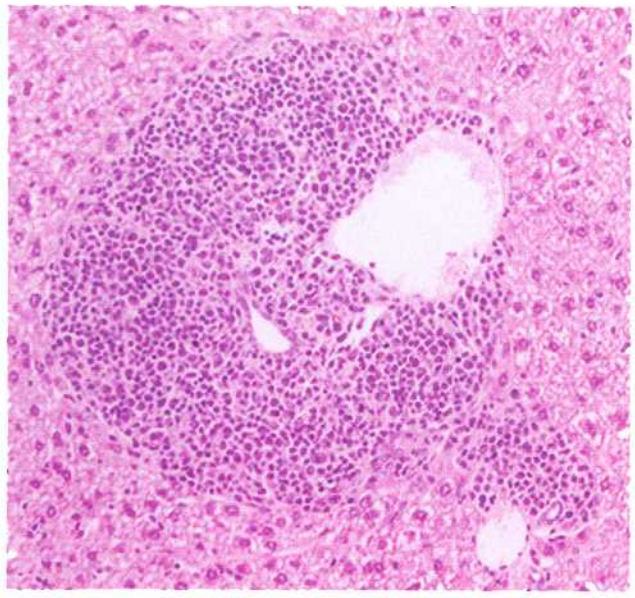


Serum level of transaminase.

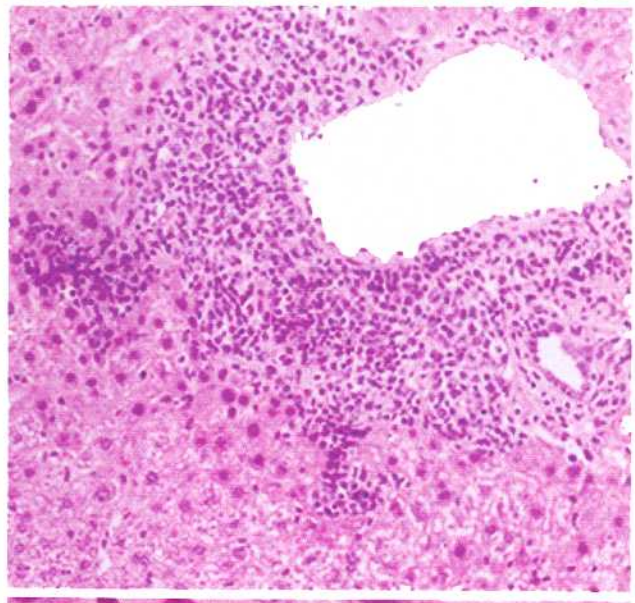
(A) AST, (B) ALT. At day 14 (9 days after concanavalon A (Con A) injection) group 4 (graft-versus-host reaction + Con A) and at day 9 group 2 (Con A) revealed the increase of serum transaminase. Results represented as Mean ± SE of each experimental group (n=8). $*P<0.05$, $**P<0.001$.

Fig. 2-3

A



B



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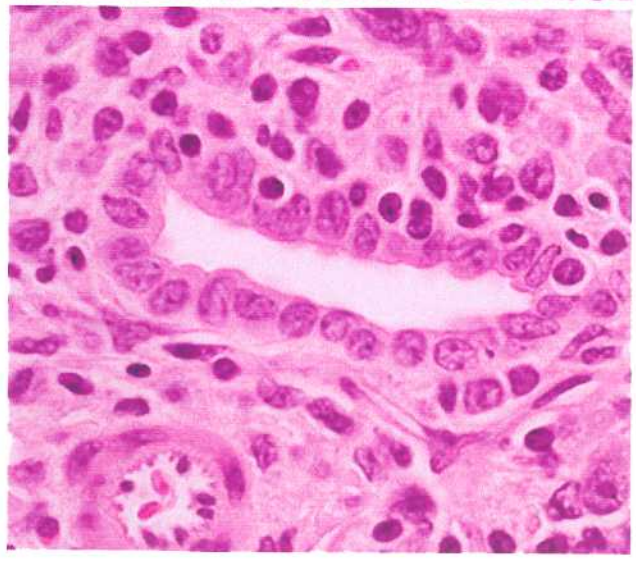
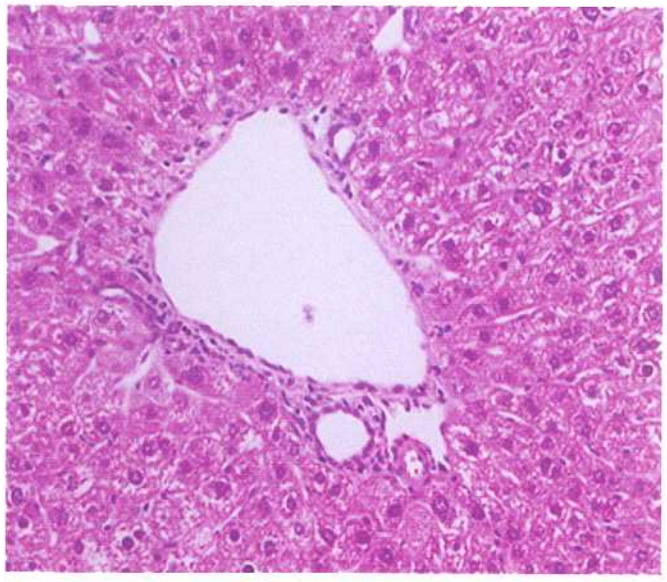
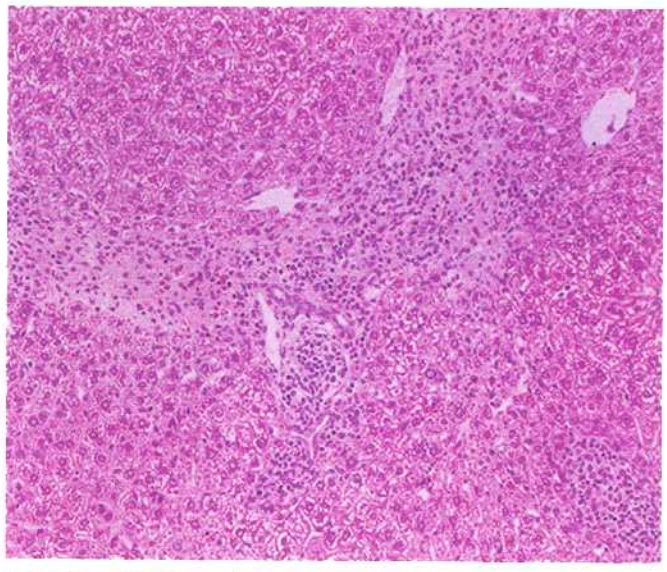


Fig. 2-3

D



E



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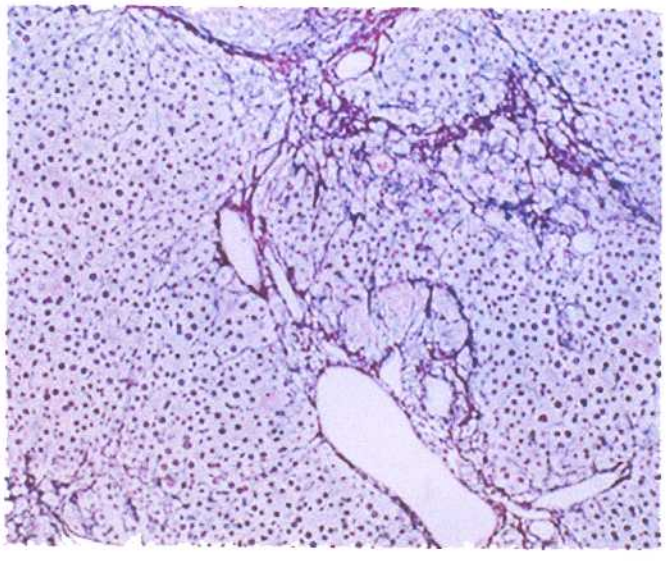


Figure 2-3 legend

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

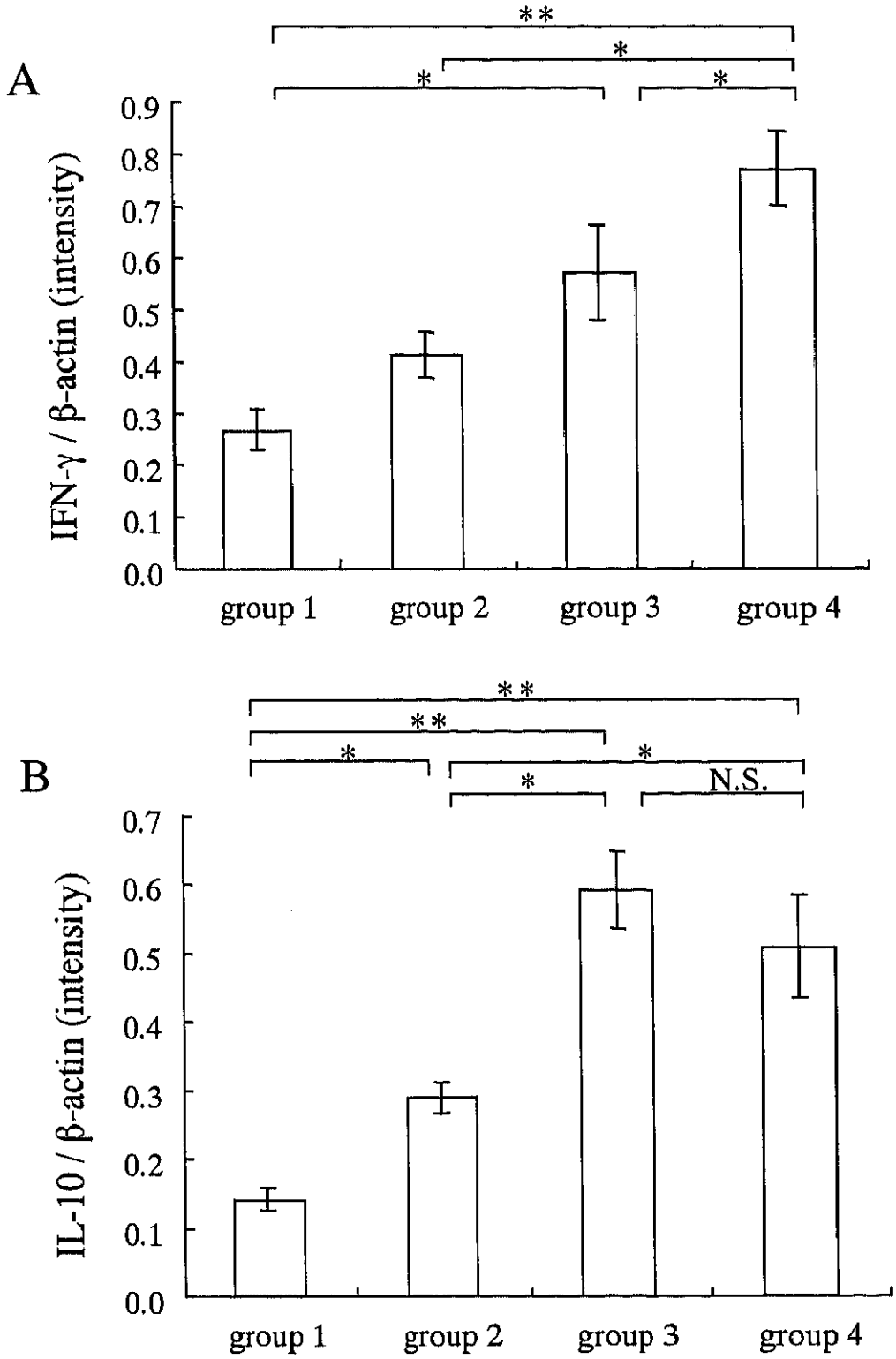

A
**
*
*
*
IFN-γ / β-actin (intensity)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
group 1
group 2
group 3
group 4
B
**
**
*
*
*
N.S.
IL-10 / β-actin (intensity)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
group 1
group 2
group 3
group 4

Figure 2-4 legend

Relative amounts of cytokine mRNA prepared from liver-infiltrating $CD4^+$ T cells.

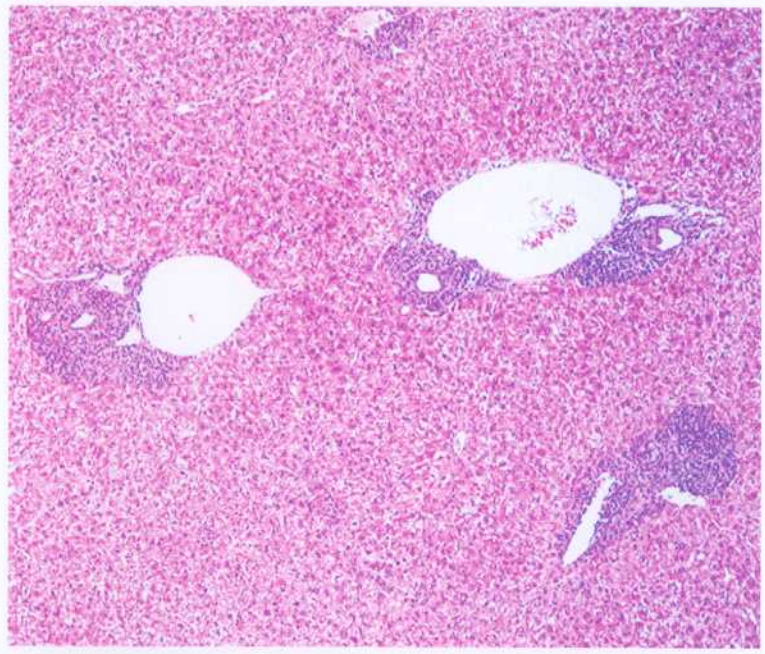
The relative amounts of fluorescence intensity from each group were measured from six independent experiments. (A) Interferom-γ / β-actin ratios were 0.27 ± 0.04 in group 1, 0.41 ± 0.04 in group 2, 0.57 ± 0.09 in group 3 and 0.77 ± 0.07 in group 4. (B) Interleukin-10 / β-actin ratios were 0.14 ± 0.01 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.

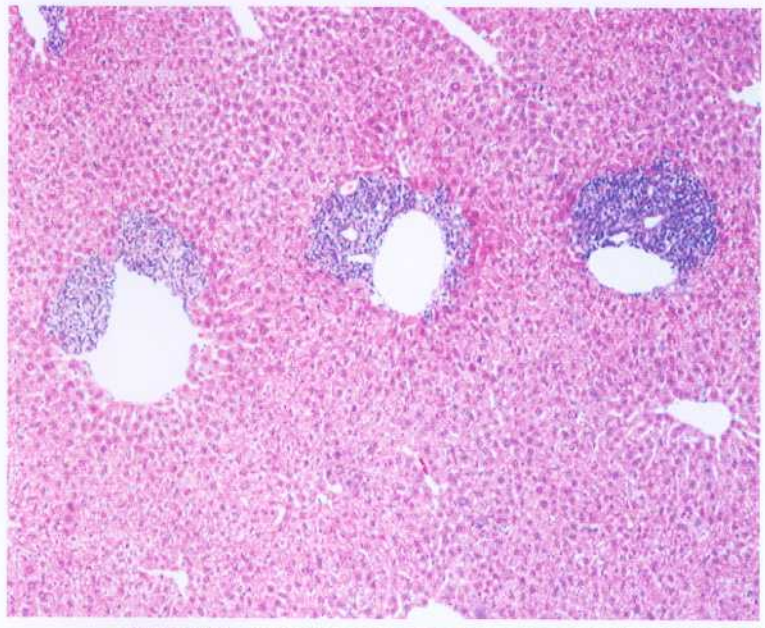
group 1: NML, group 2: concanavalin A (Con A), group 3: graft-versus-host reaction (GVHR), group 4: GVHR + Con A.

Fig. 3-1

A



B



C

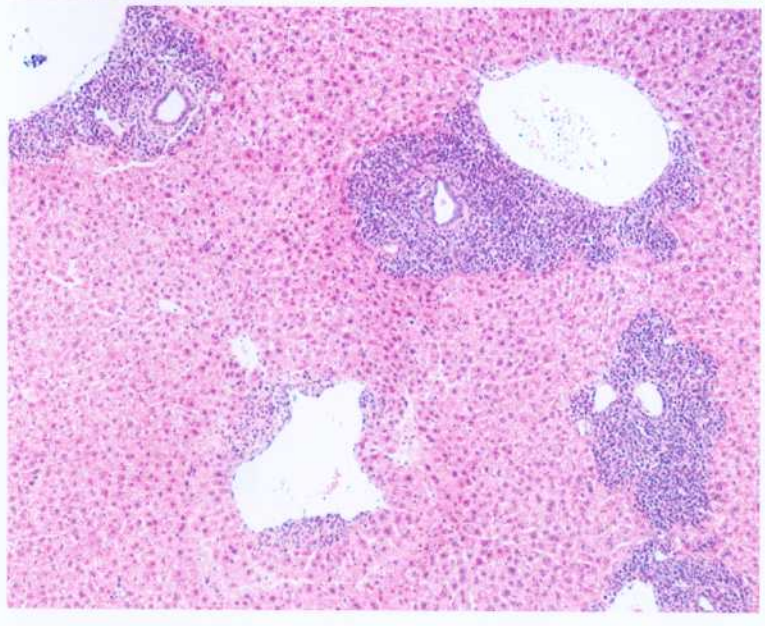
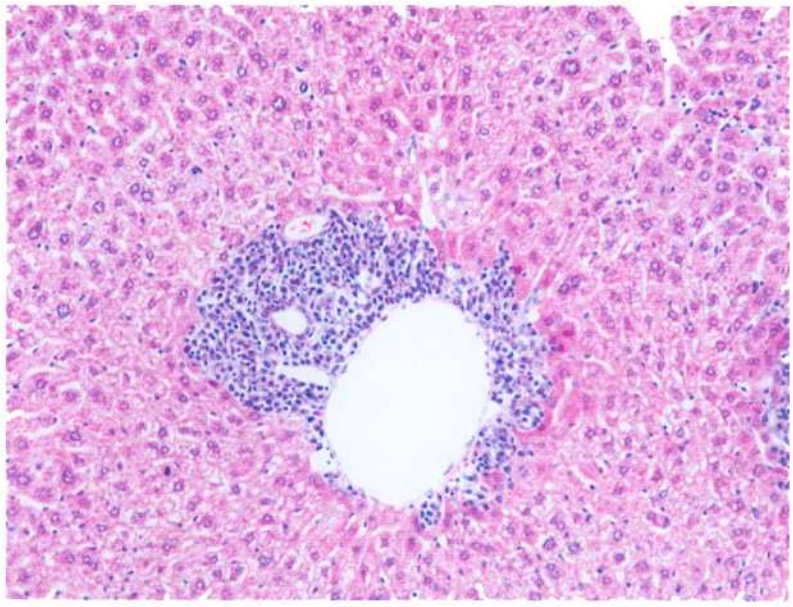


Fig. 3-1

D



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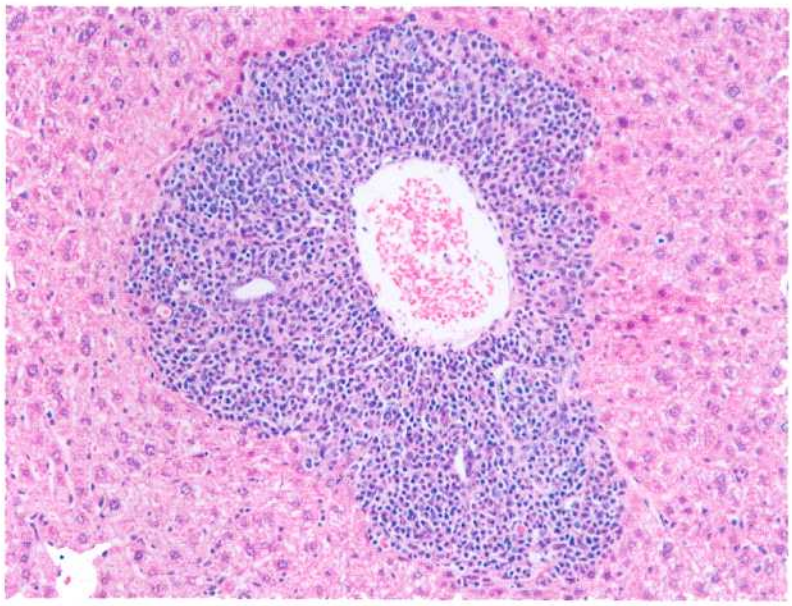

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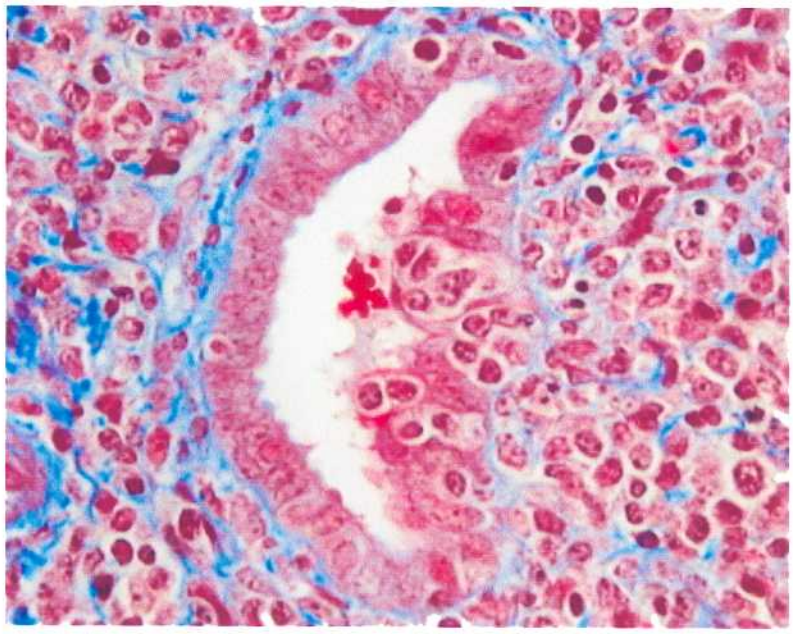

Figure 3-1 legend

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.

Figure 3-2

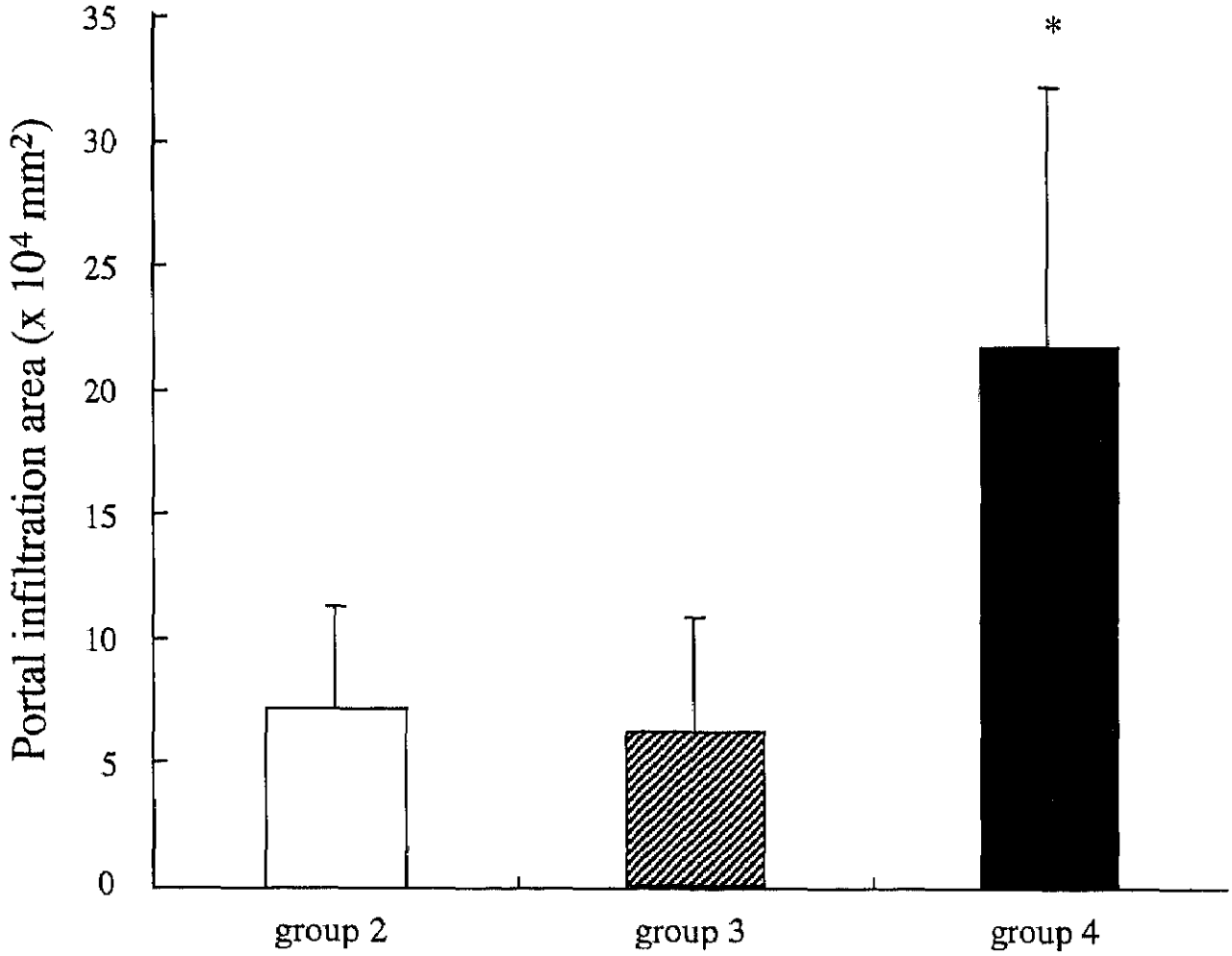

Image analysis of portal cellular infiltration area.
The area (μm^2) of infiltrated cells from five portal areas were measured for each specimen by using NIH Image. The mean of the cellular infiltrated portal area was significantly increased in group 4 (GVHR+Anti-IL-10) compared with groups 2 (GVHR) 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

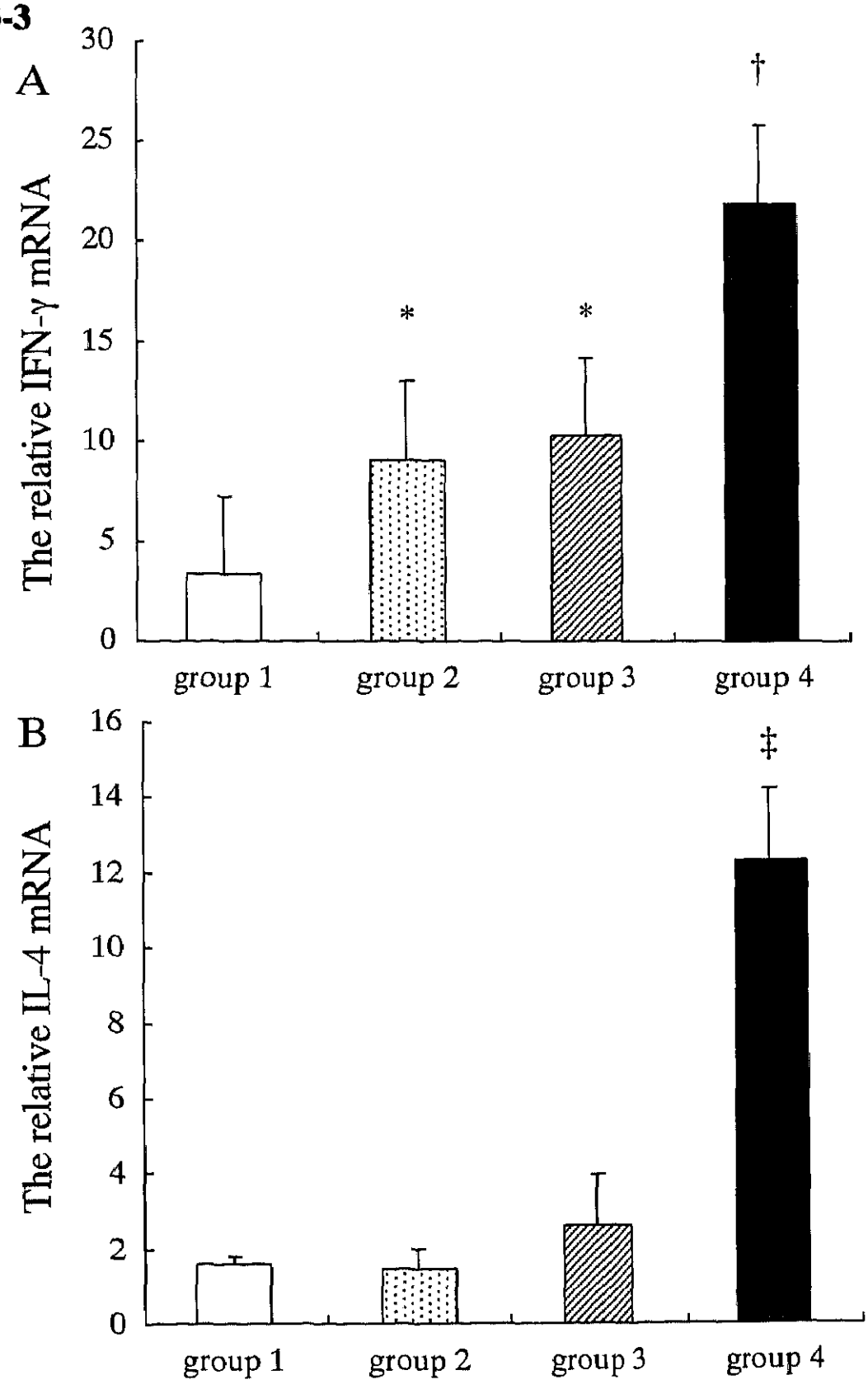
A
The relative IFN-γ mRNA
30
25
20
15
10
5
0
*
*
†
group 1
group 2
group 3
group 4
B
The relative IL-4 mRNA
16
14
12
10
8
6
4
2
0
‡
group 1
group 2
group 3
group 4

Figure 3-3 legend

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 ($\dagger P < 0.001$) and IL-4 mRNA ($\ddagger P < 0.0001$) 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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