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# A protective role of extrathymic αβ TcR cells in the liver in primary murine salmonellosis

Y. MATSUMOTO,*† M. EMOTO,† J. USAMI,*† K. MAEDA* & Y. YOSHIKAI†

**Department of Internal Medicine, Nagoya University Branch Hospital and †Laboratory of Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Japan*

## SUMMARY

The liver comprises unique T cells differentiating extrathymically and expressing an intermediate intensity of αβ T-cell receptor (TcR) and a high intensity of leucocyte function antigen-1 (LFA-1). To elucidate the functional roles of the intermediate αβ TcR cells in host defence against bacterial infection, we examined the effects of depletion of the intermediate αβ TcR cells by *in vivo* administration of monoclonal antibodies (mAb) to intercellular adhesion molecule-1 (ICAM-1)/LFA-1 and αβ TcR on the bacterial growth in the liver after infection with *Salmonella chorelaesuis* in mice. Pretreatment with mAb to LFA-1 (200 μg/mouse) together with mAb to ICAM-1 (200 μg/mouse), which could preferentially deplete the intermediate αβ TcR cells and γδ TcR cells in the liver, resulted in a severely reduced ability to resolve acute phase of *Salmonella* infection in the liver. Pretreatment with a low dose of anti-αβ TcR mAb (60 μg/mouse), which depleted only bright αβ TcR cells, did not affect the bacterial growth in the liver at the early stage after *Salmonella* infection, while the depleting of both intermediate and bright αβ TcR cells by pretreatment with a high dose of anti-αβ TcR mAb (120 μg/mouse) allowed the bacteria to multiply exaggeratedly in the liver at this stage. These results suggest that intermediate αβ TcR cells may play an important role in protection at the early stage after *Salmonella* infection in liver and that the interaction of ICAM-1/LFA-1 is critically involved in protective roles of extrathymic T cells bearing intermediate αβ TcR in liver at the early stage after *Salmonella* infection.

## INTRODUCTION

Salmonellosis is caused by Gram-negative rods, *Salmonella* species (spp.), which are facultative intracellular pathogens same as *Mycobacterium tuberculosis* and *Listeria monocytogenes*. Cellular immunity is regarded as one of the most important defence mechanisms, although humoral immunity is also involved in protection against *Salmonella* spp.[1–3] The liver is one of the target organs in murine salmonellosis.[4] Abo *et al.* have recently demonstrated that hepatic sinusoids are possible major sites for proliferation of extrathymic αβ T-cell receptor (TcR) cells.[5] Although the hepatic pathway of T-cell differentiation is relatively minor in normal mice, this pathway becomes predominant in mice under conditions of bacterial stimulation,[5] malignancies,[6,7] autoimmune diseases,[8,9] and ageing.[10] The extrathymic liver T cells have been reported to have several unique properties, including generation of forbidden T-cell oligoclones after bacterial stimulation,[5] a preponderance of $V\beta8^+$ cells,[9] expression of intermediate intensity of TcR[9] and a higher intensity of LFA-1.[11] These unique αβ TcR cells have been postulated to be important in the surveillance of bacterial infected cells and atypical cells generated *in vivo*.[12] However, there is no direct evidence that they play a role in host defence.

In the present study, to elucidate the potential roles of the unique αβ TcR cells in the liver in host defence, we investigated the effects of *in vivo* administration with monoclonal antibodies (mAb) to intercellular adhesion molecule-1 (ICAM-1)/leucocyte function antigen-1 (LFA-1) and αβ TcR on protection against *Salmonella* infection in mice. Pretreatment with mAb to ICAM-1 and LFA-1 depleted intermediate αβ TcR cells in the liver and reduced the ability to resolve infection in the liver at the early stage. Depletion of both intermediate and bright αβ TcR cells by pretreatment with a high dose of anti-αβ TcR mAb resulted in a reduced ability to deal with the acute phase of infection in the liver, but the depletion of only bright αβ TcR cells by pretreatment with a low dose of anti-αβ TcR mAb did not affect the bacterial growth at the early stage after infection. Our results suggest that the resistance in the liver at the early

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Abbreviations: HSP, heat shock protein; TLL, total liver lymphocytes.

Correspondence: Y. Yoshikai, Laboratory of Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan.

stage of primary *Salmonella* infection may be dependent mainly on a protective function of extrathymic αβ TcR cells.

## MATERIALS AND METHODS

*Animals*

Female BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan). They were fed under pathogen-free conditions. All mice were between 8 and 9 weeks of age when they were used in experiments.

*Micro-organisms*

*Salmonella choleraesuis* strain 31N-1, which is a derivative cured of a virulence plasmid of 50 kb,[13] was used in all experiments. This strain has the ability to induce the γδ TcR cells in mice.[14] The approximate intraperitoneal $LD_{50}$ of this strain for BALB/c mice was $10^7$ colony-forming units (CFU), maintained by several passages through mice. Organisms suspended in tryptic soy broth (Difco Laboratories, Detroit, MI) containing 15% glycerol (Nakarai Tesque Inc., Kyoto, Japan) were kept at −70°. The bacteria were grown in tryptic soy broth at 37° for 18 hr, harvested, washed and suspended in phosphate-buffered saline (PBS). The concentration of bacteria was quantified by plate counts. The bacteria were inoculated intraperitoneally in a dose of $1 \times 10^6$ CFU in 0·2 ml PBS.

*Antibodies and reagents*

Anti-LFA-1 α-chain (KBA), anti-ICAM-1 (YN1/1.7), anti-TcR αβ (H57-597) and anti-CD3 (145-2C11) mAb were kindly donated by Drs Y. Hashimoto and F. Takei (Tohoku University, Sendai, Japan), R. Kubo (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) and J. A. Bluestone (University of Chicago, Chicago, IL), respectively. These antibodies were obtained by growing hybridoma cells in serum-free Medium 101 (Nissui, Tokyo, Japan) and collecting supernatant. They were concentrated and purified by 50% ammonium sulphate precipitation. The purity of the preparations was confirmed by SDS–PAGE and the concentration of antibodies was determined by the Lowry method. The following were all purchased. Phycoerythrin (PE)-conjugated anti-TcR αβ mAb and FITC-conjugated anti-TcR γδ mAb were from Pharmingen (San Diego, CA). PE-conjugated anti-Thy-1.2 mAb and FITC-conjugated goat anti-hamster IgG were from Caltag Laboratories Inc. (South San Francisco, CA). FITC-conjugated goat anti-rat IgG was from Tago Inc. (Burlingame, CA). PE-conjugated anti-L3T4 mAb and biotin-conjugated anti-Lyt-2 mAb were from Becton Dickinson & Co. (Mountain View, CA). Red613-conjugated streptavidin was from Gibco BRL (Gaithersburg, MD).

*Cell preparation*

Mice were killed 3 or 6 days after the bacterial infection. Liver mononuclear cells were prepared as previously described,[15] with minor modifications. Briefly, liver was pressed through a 100-gauge stainless steel mesh after perfusion with 20 ml of Hanks' balanced salt solution (HBSS) to eliminate blood. The cell suspension was centrifuged through a 44–67·5% Percoll (Sigma Chemical Co., St Louis, MO) gradient. Cells at the interface were washed twice and used.

In vivo *administration of mAb and immunofluorescence test*

Mice were injected intraperitoneally (i.p.) with 200 μg of KBA together with 200 μg of YN1/1.7, or 60 μg or 120 μg of H57-597, diluting to a final volume of 200 μl in PBS 4 days before inoculating with *S. choleraesuis* strain 31N-1. In a control group, 200 μl of PBS was injected. To examine the effect of *in vivo* administration of mAb on the surface antigen of T cells, prepared liver mononuclear cells were subjected to cytofluorometric analysis on day 0 before infection or day 3 or 6 after infection. For sharp detection of a αβ T-cell subpopulation,[11] two-colour staining of αβ TcR and LFA-1 antigens was used. Other surface phenotypes of cells were identified by using various mAb in conjunction with the single- to three-colour staining. The fluorescence-positive cells were analysed by a FACScan (Becton Dickinson & Co.).

*Bacterial growth in liver and spleen*

Mice were killed by cervical dislocation 3, 6 or 10 days after infection. The bacterial count was carried out according to the method previously described,[16] with minor modification. Briefly, organs were removed aseptically to homogenizer tubes containing 3 ml (for liver) or 4 ml (for spleen) of PBS, giving final volumes of approximately 5 ml. They were dispersed for 1 min with a Teflon homogenizer. The viable bacterial count of the viscera was estimated from colony counts after a 48-hr culture at 37°. The medium used for culture was tryptic soy agar (Difco Laboratories, Detroit, MI). The detection limit of this procedure was 50 CFU of *S. choleraesuis* per organ.

*Statistical analysis*

Each experimental group consisted of four mice. For each group, data were analysed by one-way analysis of variance with the Mann–Whitney *U*-test. Values of $P < 0.05$ were considered statistically significant.

## RESULTS

### Effect of pretreatment with YN1/1.7 and KBA mAb on the eradication of bacteria in mice infected with *S. choleraesuis*

A sublethal dose ($1 \times 10^6$) of viable *S. choleraesuis* was injected i.p. in four mice 4 days after pretreatment with mAb. The kinetics of bacterial growth in the liver and spleen were monitored for 10 days after infection. In accordance with our earlier findings,[14] the numbers of viable bacteria in the liver decreased linearly with time in control mice pretreated with PBS. As shown in Fig. 1 and Fig. 2, the number of bacteria in the liver of mice pretreated with YN1/1.7 and KBA on day 3 after infection was significantly greater than that in control group ($P < 0.05$), although no difference in bacterial number was evident between both groups on day 6 and day 10 after infection. These results suggested that the bacterial clearance in liver on day 3 after infection was significantly inhibited by *in vivo* pretreatment with mAb to ICAM-1 and LFA-1.

### Effect of pretreatment with H57-597 mAb on the eradication of bacteria in mice infected with *S. choleraesuis*

The kinetics of bacterial growth were examined in liver and spleen of four mice pretreated with 60 μg or 120 μg of H57-597

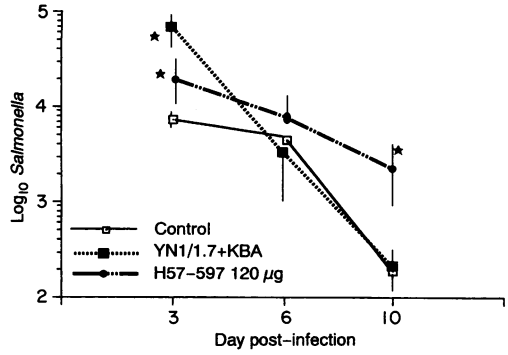


**Figure 1.** Effect of pretreatment with anti-ICAM-1 (YN1/1.7) plus anti-LFA-1 (KBA) mAb or anti-TcR αβ (H57-597) mAb on the bacterial growth of *S. choleraesuis* in the liver. Female BALB/c mice were injected intraperitoneally with both 200 μg of YN1/1.7 and 200 μg of KBA, 120 μg of H57-597 or 200 μl of PBS as control, and 4 days later inoculated with $1 \times 10^6$ CFU of *S. choleraesuis* strain 31N-1. Data are means ± SD of four mice per group per time-point. $*P < 0.05$. Statistical significance was determined by Mann–Whitney *U*-test.

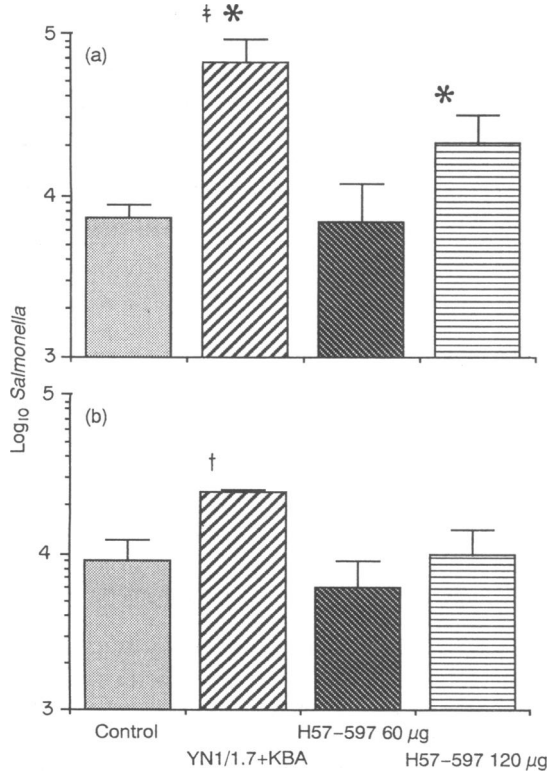


**Figure 2.** Evidence that pretreatment with anti-ICAM-1 (YN1/1.7) plus anti-LFA-1 (KBA) mAb or anti-TcR αβ (H57-597) reduced the capacity of resolving infection in the liver (a) or spleen (b) on day 3 after inoculation with *S. choleraesuis*. Female BALB/c mice were injected intraperitoneally with both 200 μg of YN1/1.7 and 200 μg of KBA, or 60 μg or 120 μg of H57-597, and 4 days later inoculated with $1 \times 10^6$ CFU of *S. choleraesuis*. Data are means ± SD of four mice per group. $*P < 0.05$, $\dagger P < 0.01$, significantly different from values for control mice. $\ddagger P < 0.05$, significantly different from values for mice treated with 120 μg of H57-597.

after inoculation of viable *Salmonella*. The number of bacteria on day 3 after infection was greater in liver of mice pretreated with 120 μg of H57-597 than those of mice treated with 60 μg of H57-597 and control mice ($P < 0.05$), while there was no significant difference in the spleen on day 3 after infection among three groups (Figs 1 and 2). In contrast with mice pretreated with YN1/1.7 plus KBA, the number of bacteria from H57-597-pretreated mice was greater than that from the control group in liver ($P < 0.05$, Fig. 1) and spleen (data not shown) on day 10 after infection. Thus, bacterial clearance after ~6 day of *Salmonella* infection was prevented by *in vivo* administration of mAb to αβ TcR, consistent with our previous reports showing that an appreciable number of bacteria persisted in spleen of H57-597-treated mice at the late stage during listeriosis.[17] When comparing the number of bacteria in the liver between mice pretreated with 120 μg of H57-597 and those treated with YN1/1.7 and KBA, it was noticeable that the number in the latter mice was greater than in the former mice on day 3 after *Salmonella* infection ($P < 0.05$).

### Effect of *in vivo* treatment with anti-ICAM-1 plus LFA-1 mAb on expression of ICAM-1 or LFA-1 or αβ TcR on liver lymphocytes

We examined surface expression on liver lymphocytes 4 days after administration of mAb (on day 0 before infection). As shown in Fig. 3a, the intensity of expression of LFA-1 molecules decreased from 554 fluorescence peak to 28 after *in vivo* administration of anti-ICAM-1 and LFA-1 mAb, and a similar pattern was observed in the expression of ICAM-1 molecules (data not shown). Consistent with previous reports,[9] single-colour analysis of αβ TcR revealed that liver contained two types of αβ TcR cells: high intensity and intermediate intensity αβ TcR. As shown in Fig. 3b, *in vivo* administration of anti-ICAM-1 and LFA-1 mAb led to the disappearance of the intermediate peak of αβ TcR. These results indicated that ICAM-1 and LFA-1 molecules on T cells were clearly down-regulated and the appearance of intermediate αβ TcR cells with high intensity of LFA-1 molecules severely suppressed by *in vivo* administration of mAb to ICAM-1 and LFA-1.

Next we examined T-cell subpopulations in the liver of mice pretreated with YN1/1.7 and KBA mAb on day 3 after infection, by two-colour FACS analysis for expression of LFA-1 and αβ TcR, or of Thy-1.2 and γδ TcR. A typical finding is shown in Fig. 4, and the mean absolute numbers of total liver lymphocytes (TLL) and T-cell subsets of four mice of each group are summarized in Table 1. By two-colour staining of LFA-1 and αβ TcR, the existence of intermediate αβ TcR cells was easily detectable, as indicated by the squares (Fig. 4). This was due to the relatively higher expression of LFA-1 on intermediate αβ TcR cells than on bright αβ TcR cells. There was no significant difference in the absolute number of TLL between control mice and mice treated with YN1/1.7 plus KBA. The intermediate αβ TcR cells were 11–14% of TLL in infected control mice, while the population derived from mice treated with YN1/1.7 and KBA constituted ~4% of TLL. In contrast, the percentage of bright αβ TcR cells was not affected in mice pretreated with YN1/1.7 and KBA (Table 1). Consistent with our previous report,[14] γδ TcR cells in the control group increased significantly from $0.8 \times 10^5$ before infection to $3.1 \times 10^5$ on day 3 after infection, while γδ TcR cells in mice treated with YN1/1.7 and KBA increased only marginally ($1.7 \times 10^5$). These results

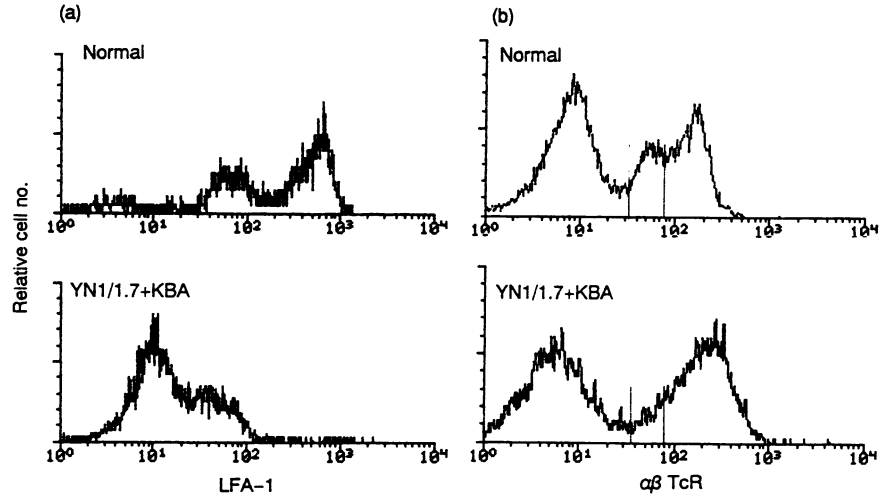


**Figure 3.** Effect of *in vivo* treatment with anti-ICAM-1 (YN1/1.7) plus LFA-1 (KBA) mAb on expression of LFA-1 (a) or αβ TcR (b) on liver lymphocytes. Liver lymphocytes were isolated from mice 4 days after administration of 200 μg of YN1/1.7 and 200 μg of KBA, and single-cell suspensions were labelled with PE-conjugated anti-TcR αβ mAb and anti-LFA-1 mAb followed by FITC-conjugated goat anti-rat IgG.

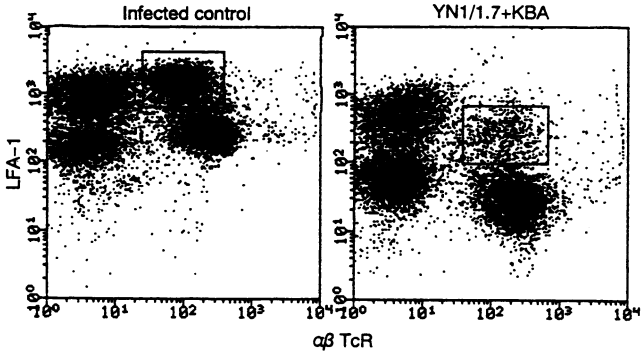


**Figure 4.** Effect of pretreatment with anti-ICAM-1 (YN1/1.7) plus anti-LFA-1 (KBA) mAb on T-cell subsets in the liver on day 3 *Salmonella*-infected mice. Mice received injections of 200 μg of YN1/1.7 and 200 μg of KBA 4 days before infection, and were killed on day 3 after infection. Liver lymphocytes were isolated and single-cell suspensions were labelled for two-colour flow cytometry with PE-conjugated anti-TcR αβ mAb and anti-LFA-1 mAb followed by FITC-conjugated goat anti-rat IgG. Intermediate αβ TcR cells are indicated by squares.

suggest that the early appearance of not only T cells bearing intermediate-αβ TcR but also γδ TcR in mice may be suppressed by *in vivo* pretreatment with both YN1/1.7 and KBA because of down-regulation of expression of ICAM-1 and LFA-1 molecules on the lymphocytes.

### Effect of pretreatment with H57-597 mAb *in vivo* on T-cell subpopulations in the liver of mice

T-cell subpopulations were also examined in mice pretreated with H57-597 mAb before and on day 3 after *Salmonella* infection. *In vivo* administration of a low dose of H57-597 (60 μg/mouse) led to depletion of bright, but not intermediate αβ TcR cells, while that of high dose of H57-597 (120 μg/mouse) led to depletion of both bright and intermediate αβ TcR cells before (data not shown, ref. 17) and on day 3 after infection (Fig. 5). The T cells were hardly stained with FITC-conjugated anti-hamster IgG only, excluding the possibility that αβ TcR on T cells were merely occupied by anti-TcR αβ mAb (data not shown). These results indicated that bright αβ TcR cells are more susceptible to depletion than intermediate αβ TcR cells by *in vivo* administration of mAb to αβ TcR. On the other hand, the proportion of γδ TcR cells derived from mice pretreated with 60 μg or 120 μg of H57-597 was significantly increased on day 3 after infection compared with that from infected control mice ($P < 0.05$, Table 1), and the absolute number of γδ TcR cells was larger in mice pretreated with 120 μg of H57-597 mice than that in mice pretreated with KBA and YN1/1.7 ($P < 0.05$, Table 1).

## DISCUSSION

In the present study, we have obtained the first evidence for the protective roles of the unique liver T cells, which differentiate extrathymically and bear intermediate intensity of αβ TcR and large amounts of LFA-1 molecules, in bacterial infection. Mice depleted of intermediate αβ TcR cells in liver by pretreatment with anti-ICAM-1 and anti-LFA-1 mAb or by pretreatment with a high dose of anti-TcR αβ mAb had significantly reduced ability to stabilize *Salmonella* infection in the liver at the early stage. On the other hand, pretreatment with a low dose of anti-TcR αβ mAb, which only depleted bright αβ TcR cells in the liver, did not affect the bacterial growth at the early stage after *Salmonella* infection. Taken together, intermediate αβ TcR cells appear to play an important role in protection at the early stage after *Salmonella* infection in the liver.

Abo *et al.* have reported that bacterial stimulation such as an i.p. injection of heat-killed *Propionibacterium acnes* or *Escherichia coli* induced intermediate αβ TcR cells with a $CD4^-$ $CD8^-$ phenotype in the liver which differentiated along an extrathymic pathway,[5] suggesting that the unique T cells of the liver may play some roles in host defence at the early stage after bacterial

**Table 1.** Absolute and relative numbers of total liver lymphocytes and subpopulations derived from day 3 *Salmonella*-infected mice

| Treatment | No. of liver lymphocytes × $10^5$ (%) | | | |
|---|---|---|---|---|
| | All cells | Intermediate αβ T | Bright αβ T | γδ T |
| Infected control | 31·9±3·7 | 3·9±1·0 (12·5) | 7·1±0·8 (22·2) | 3·1±0·8 (9·7) |
| YN1/1.7+KBA | 26·0±7·6 | 0·9±0·3 (3·4) | 7·3±1·1 (28·0) | 1·7±0·4 (6·5) |
| H57-597 60 μg | 19·0±5·4 | 3·9±1·3 (20·5) | 1·2±0·3 (6·3) | 3·1±0·3 (16·3)† |
| H57-597 120 μg | 24·7±7·2 | 0·2±0·1 (0·8) | <0·1 | *4·2±1·1 (17·0)† |

Monoclonal antibodies were given 4 days before infection with 1 × $10^6$ CFU *S. choleraesuis*. Liver lymphocytes were isolated from mice on day 3 after infection. Viable cell counts were determined and single-cell suspensions were subjected to cytofluorometric analysis. Absolute numbers represent means ± SD of four mice per group. Percentages are means of four mice per group.

*$P < 0.05$, significantly different from values for mice treated with YN1/1.7 and KBA.

†$P < 0.05$, significantly different from values for control mice.

infection. The findings presented here confirm their hypothesis for protective roles of the unique liver T cells in host defence.

The adhesive interaction of cells with other cells has an important role in the functions of the immune system.[18] In adhesion molecules, LFA-1 and ICAM-1, a counter receptor for LFA-1, play an important role in adhesion of T cells to vascular endothelium, antigen-presenting cells and target cells in cytotoxic reactions.[19,20] In the presence of infectious organisms, the first step in localization of immunocompetent cells is binding of lymphocytes and monocytes to endothelium, on which surfaces ICAM-1 molecules are strongly induced by inflammatory mediators including interleukin-1, interferon-γ and tumour necrosis factor.[19,20] Therefore, our finding for suppressive effects of mAb to ICAM-1/LFA-1 on protection against *Salmonella* infection may account for inhibition of early influx of immunocompetent cells in the liver of mice pretreated with these mAb. However, the absolute number of lymphocytes in mice pretreated with mAb to ICAM-1/LFA-1 did not differ from those in control mice, suggesting that infiltration and accumulation of the leucocytes may not be affected in these mice. ICAM-1/LFA-1 interaction can function as a potent costimulatory signal to antigen-specific T-cell proliferation because the cross-linking of LFA-1 and CD3 simultaneously enhances both interleukin-2 production and proliferation of T cells.[21] Recent studies on inhibition of allograft rejection by treatment with mAb to ICAM-1 and LFA-1[22] implicate their interaction as essential for generation of T cells responsible for allograft rejection. Taken together, we speculate that proliferation or activation of intermediate αβ TcR cells at the early stage after infection may be prevented by mAb to ICAM-1/LFA-1 molecules. Our results revealed that *in vivo* administration of anti-ICAM-1/LFA-1 mAb did not affect the protection at the late stage after infection with *S. choleraesuis* (Fig. 1). *In vivo* pretreatment with mAb to ICAM-1 (200 μg/mouse) and LFA-1 (200 μg/mouse) for 1 day may not be sufficient to inhibit the generation of *Salmonella*-specific bright αβ TcR cells at the late stage after infection. Alternatively, ICAM-1/LFA-1 interaction may contribute less to the generation of αβ TcR cells responsible for protection at the late stage after infection.

*In vivo* pretreatment with a high dose of anti-TcR αβ mAb suppressed the protective mechanism at the late stage as well as at the early stage after infection (Fig. 1), suggesting that bright αβ TcR cells may play important roles in protection at the late stage after infection, as reported previously in murine listeriosis.[17] *In vivo* treatments with anti-TcR αβ mAb are reported to often stimulate the αβ TcR cells to produce cytokines which

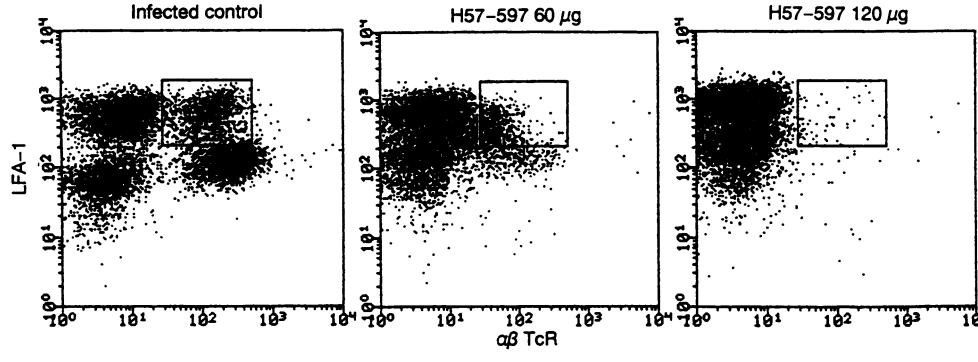


**Figure 5.** Effect of pretreatment with anti-TcR αβ (H57-597) mAb on T-cell subsets in the liver on day 3 *Salmonella*-infected mice. Mice received injections of 60 μg or 120 μg of H57-597 4 days before infection, and were killed on day 3 after infection. Liver lymphocytes were isolated and single-cell suspensions were labelled for two-colour flow cytometry with PE-conjugated anti-TcR αβ mAb and anti-LFA-1 mAb followed by FITC-conjugated goat anti-rat IgG. Intermediate αβ TcR cells are indicated by squares.

modify immune responses. So far, we cannot rule out the possibility that stimulation of anti-TcR αβ mAb may lead to release of cytokine and consequently inhibit immune effective mechanisms. However, our results with staining with FITC-conjugated anti-hamster IgG only suggest that a significant fraction of αβ TcR cells was definitely deleted. Furthermore, suppressive effects of a high dose of anti-TcR αβ mAb on protection at the early stage after *Salmonella* infection was detected in liver but not in spleen, excluding the possibility for stimulating effects of anti-TcR αβ mAb.

A question is raised as to how intermediate αβ TcR cells of the liver associate with early protection in salmonellosis. The intermediate αβ TcR cells in liver have been reported to include potentially autoreactive T cells, estimated by Vβ analysis.[5,9] While the autoreactive αβ TcR cells in the liver may be rendered tolerant by clonal anergy,[23] there has been an evidence that infection breaks an established T-cell tolerance induced by injection with *Staphylococcus enterotoxin* B.[24] Therefore, it is possible that these T cells, including dominantly autoreactive T cells, may be activated by the bacterial infection. Alternatively, it is possible that the intermediate αβ TcR cells may proliferate in response to phylogenetically conserved antigens such as heat shock protein (HSP) expressed by infected cells. Whatever the T cells recognize, an appropriate generation of extrathymic αβ TcR cells will be beneficial for host defence at the early stage before the highly evolved type of immunity by bright αβ TcR cells is induced.

We have previously demonstrated that the early appearing γδ TcR cells during salmonellosis may serve as a first defence in the liver and peritoneal cavity. Our results showed that mice pretreated with anti-ICAM-1 and LFA-1 mAb exhibited the more severely reduced ability to stabilize infection at the early stage compared with mice pretreated with a high dose of anti-TcR αβ mAb. It was noticeable that γδ TcR cells derived from the latter mice were increasing compared to those from the former mice. It seems reasonable to suggest that γδ TcR cells as well as intermediate αβ TcR cells are involved in early resistance against bacterial infection, and ICAM-1 and LFA-1 adhesion may be critically involved in protective roles of both T cells in liver at the early stage of *Salmonella* infection.

In conclusion, we show direct evidence that intermediate αβ TcR cells in the liver of mice function properly in host defence against *Salmonella* infection. We have obtained evidence for a protective role of γδ TcR cells in liver at the early stage after infection *S. choleraesuis*.[14] The protective immune response in the liver seems to require the combined efforts of a number of mechanisms, including extrathymic αβ, γδ TcR cells and Kupffer cells of hepatic sinusoids. Further investigation of the relative contribution of lymphocyte subpopulations and sinusoidal cells will account for the unique defence mechanism of liver.

## ACKNOWLEDGMENTS

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