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REGULATORY ROLE OF HEAT SHOCK PROTEIN-SPECIFIC T CELLS IN HOST DEFENSE

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ABSTRACT

During infection with *L.monocytogenes*, a facultative intracellular bacteria, TcR γ/δ T cells specific for 65 kd hsp precede TcR α/β T cells specific for the listerial antigens in appearance. The γ/δ T cells provide a first line of defense against the infection by recognizing exogenous and endogenous 65 kd hsp on infected cells and producing cytokines such as γIFN. The hsp-specific T cells respond quickly to antigenically diverse pathogens before antigen-specific T cells expand clonally, and they play a role in covering the gap between the phagocytic system and highly evolved immune response. 65 kd hsp-specific T cells play important roles not only in host defense mechanism against infection with various pathogens but also in induction of autoimmune disease. Both 65 kd hsp-specific γδ T cells and 65 kd hsp-specific αβ T cells abrogate the unresponsiveness of the self-reactive αβ T cells and/or B cells by producing IL-2 and contribute to induction of autoimmune disease.

Key Words: γδ T cells, αβ T cells, 65 kd HSP, *Listeria monocytogenes*, Autoimmune disease

INTRODUCTION

Heat shock proteins (hsp), polypeptides phylogenically conserved from prokaryotes to eukaryotes, are frequently the targets of humoral and cell-mediated immune responses during infection and autoimmune diseases.[1-5] Among the various kinds of hsp, the 65 kd (kilo dalton) mycobacterial hsp has been identified as a target of T cells capable of causing autoimmune diseases in a rat model of adjuvant-induced arthritis,[6] in the synovial infiltrates of rheumatoid arthritis patients,[7] and in leprosy and tuberculosis.[8] Although most of the hsp-specific T cells express T cell receptor (TcR) α/β, there have been several lines of evidence that at least a substantial fraction of T cells bearing TcR γ/δ are specialized to recognize mycobacterial antigens including 65 kd hsp.[9-12]

In the present study, we focus on the roles of hsp-specific T cells in host defense against infection with a facultative intracellular bacteria, *Listeria monocytogenes*, and in induction of autoimmune diseases in mice. We have found that TcR γ/δ-bearing T cells capable of recognizing 65 kd hsp contribute to the first line of the host defense against primary infection with *L.monocytogenes* through production of γ interferon (IFN) and that hsp-specific T cells, regardless of their TcR being α/β or γ/δ, play an important role in the induction of autoimmune diseases in aged nude mice and newborn-thymectomized (NTX) mice.

THE ROLE OF HSP-SPECIFIC γ/δ T CELLS IN HOST-DEFENSE AGAINST INFECTION WITH L.MONOCYTOGENES

Murine host response to *L.monocytogenes* has been a useful model for studying protective immunity against facultative intracelluar pathogens.[13] The *Listeria*-immune T cells and their related cytokines represent the major mediators that confer protection against the disease. Recently, at least a substantial fraction of γ/δ T cels are reported to recognize common epitopes of 65 kd hsp derived from various bacteria and mammalian cells.[9,12] To elucidate a potential role of the hsp-reactive γ/δ T cells in host defense against *Listeria*, we examined the kinetics, V repertoire, specificity and functions of γ/δ T cells during an intraperitoneal infection with *L.monocytogenes*.

The number of *Listeria* in the organs peaked on day 3 and gradually decreased by day 8 after infection. $CD3^+CD4^-CD8^-$ cells bearing TcR γ/δ in the peritoneal cavity, increased in proportion on day 3 and thereafter decreased by day 8 after infection, whereas $CD3^+CD4^+CD8^-$ cells or $CD3^+CD4^-CD8^+$ cells bearing TcR α/β increased by day 8 after infection (Fig.1). These results suggested that the γ/δ T cells precede the α/β T cells in appearance during listerial infection. PCR analysis with TcR Vγ and Vδ primers revealed that the early-appearing γ/δ T

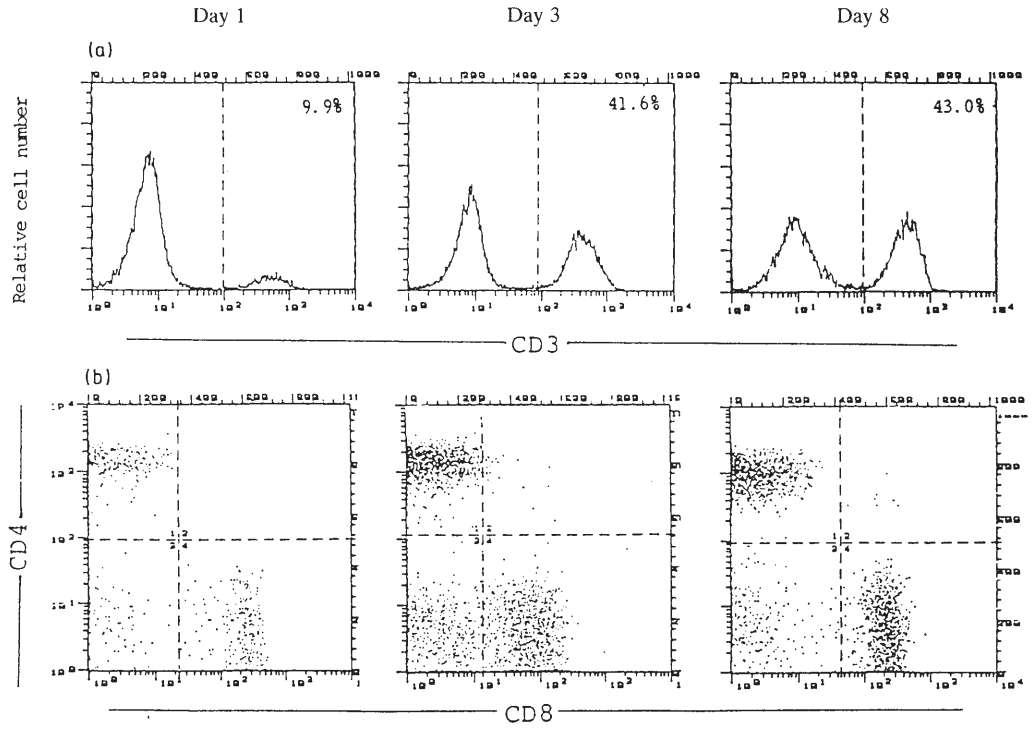


Fig. 1. Three-color FACS analysis of CD3, CD4 and CD8 expression in the nonadherent population of PEC obtained from mice after i.p. inoculation with *L.monocytogenes*. Nonadherent population from the PEC on day 1, 3 and 8 after i.p. injection of 1×10^3 *L.monocytogenes* was stained with FITC-anti-CD3 and PE-anti-CD4 mAb and biotin-CD8 mAb, then with DuoCHROME-conjugated streptavidin.
(a) Relative cell number of $CD3^+$ was presented on day 1,3 and 8 after the infection.
(b) The profile of PE-CD4 and DuoCHROME-CD8 was displayed after gating on the $CD3^+$ cells using forward light scatter to exclude dead cells and red blood cells.

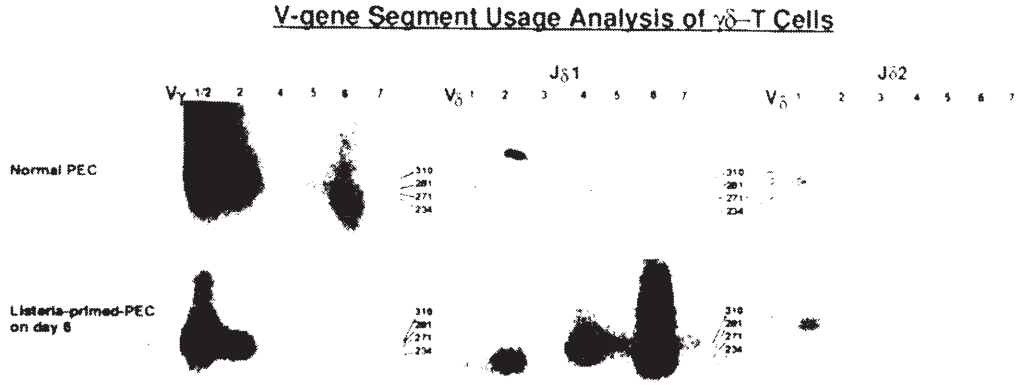


Fig. 2. V-gene segment usage analysis of γδ-T cells. RNA were prepared from peptone-induced PEC from normal mice or *Listeria*-infected mice. RNA were primed either with 10 pmol γ chain C region primer or δ chain C region primer in 20 μl reaction mixture for reverse transcription. The PCR was performed on a program temp. control system PC-500 (ASTEC, Japan). The 5' primers are as follows: Vγ1/2 ACACAGCTATACATTGGTAC, Vγ2 CGGCAAAAAACAAATCAACAG, Vγ4 TGTCCTTGCAACCCCTACCC, Vγ5 TGTGCACTGGTACCAACTGA, Vγ6 GGAATTCAAAAGAAAACATTGTCT, Vγ7 AAGCTAGAGGGGTCCTCTGC, Vδ1 ATTCAGAAGGCAACAATGAAAG, Vδ2 AGTTCCCTGCAGATCCAAGC, Vδ3 TTCCTGGCTATTGCCTCTGAC, Vδ4 CCGCTTCTCTGTGAACTTCC, Vδ5 CAGATCCTTCCAGTTCATCC, Vδ6 TCAAGTCCATCAGCCTTGTC, Vδ7 CGCAGAGCTGCAGTGTAACT. One tenth of each γ and δ-PCR products were electrophoresed on 1.5% agarose gel and transferred to Gene Screen Plus. The southern blots of γ- and δ-PCR products were hybridized with ^{32}P-labelled γ or δ chain Jδ1 or Jδ2 probe.

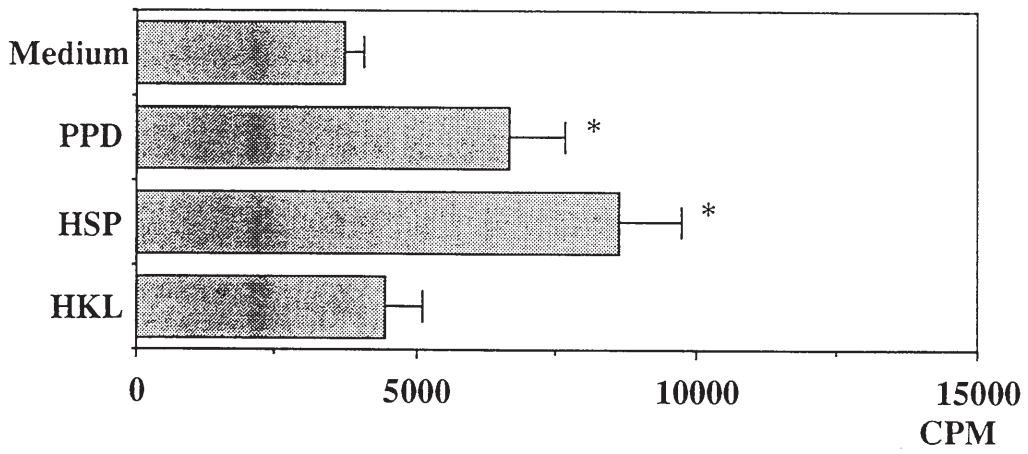


Fig. 3. Proliferation of γδ-T cells stimulated with various antigens. *In vitro* stimulation of peritoneal γδ-T cells in *Listeria*-infected mice with mycobacterial antigens. PEC on day 6 were passed through nylon-wool and both CD4 and CD8 cells were depleted by treatment with anti-CD4 mAb, anti-CD8 mAb and complement. Proliferative responses to PPD, HSP or HKL were measured by 12-h ^{3}H-thymidine incorporation in cells (3×10^5/well) cultured for 72 h in the presence of irradiated (3000R) syngeneic spleen cells (3×10^5/well). The counts per minute (c.p.m.) for ^{3}H-thymidine incorporation were measured in triplicated cultures. The results are mean ± SD.

*$p < 0.005$ when compared with the medium-alone control

cells preferentially used V γ1/Vδ6, which is reported to be often used by 65 kd hsp-specific γ/δ T cells (Fig.2).[14] To determine the possible ligands for the early-appearing γ/δ T cells during listeriosis, a γ/δ T cell-enriched population in the PEC was cultured with heat-killed *Listeria*, PPD or 65 kd hsp. A γ/δ T cell-enriched population was obtained by treatment of PEC passed through nylon-wool, with anti-CD4 mAb, anti-CD8 mAb and complement. As shown in Fig.3, the γ/δ T cells significantly responded to PPD and 65 kd hsp in the presence of syngeneic irradiated spleen cells, while the γ/δ T cells did not proliferate in response to heat-killed *Listeria*. The cytokine production was examined in the supernatants of 3-day culture of γ/δ T cells with PPD/65 kd hsp. A significant level of γIFN was detected in the supernatants of the culture of γ/δ T cells with 65 kd hsp, whereas IL-2 activity in the supernatants was hardly detected (Table 1). These results suggest that the early-appearing γ/δ T cells during listerial infection are specialized to recognize 65 kd hsp and produce γIFN.

Table 1. Cytokine Production of Listeria-primed γδ-T Cells Stimulated with Various Antigens

Antigen	γ-INF	IL-2
PPD	1680 U/ml	undetectable*
HSP	940	undetectable
HKL	33	undetectable
Medium	66	undetectable

To further investigate the protective role of γ/δ T cells in listerial infection, TcR α/β T cell-depleted mice or TcR γ/δ T cell-depleted mice were prepared by *in vivo* treatment with anti-TcR α/β mAb or anti-TcR γ/δ mAb, respectively. We confirmed with FCM analysis that the number of TcR α/β T cells or TcR γ/δ T cells remained at an undetectable level in the peripheral lymphoid tissues by day 8 after an intraperitoneal injection of 100 μg purified anti-TcR α/β mAb or anti-TcR γ/δ mAb. A sublethal dose of viable *Listeria* was injected into the peritoneal cavity of mice three days after treatment with anti-TcR α/β mAb or anti-TcR γ/δ mAb, and the kinetics of bacterial growth were examined at various intervals after infection. As shown in Fig.4a, the number of *Listeria* in the spleen of TcR α/β T cell-depleted mice on day 3 after infection was much the same as that of untreated mice at this stage, while a significantly increased number of *Listeria* were detected in TcR γ/δ T cell-depleted mice as compared with those in TcR α/β T cell-depleted mice and untreated mice at this stage after infection. The number of bacteria in the untreated mice decreased to an undetectable level by day 8 after infection. On the other hand, an appreciable number of bacteria remained in TcR α/β mAb-treated mice on day 8 after infection in spite of the presence of TcR γ/δ T cells. On the other hand, *Listeria* were completely eliminated in TcR γ/δ T cell-depleted mice at this stage (Fig.4b). These results suggest that the early- appearing γ/δ T cells may participate in host defense at the early stage of listerial infection. The notable findings in the present study are as follows:

1) The γ/δ T cells precede the α/β T cells in appearance in the peritoneal cavity during an intraperitoneal infection with *Listeria*.
2) The early-appearing γ/δ T cells display a limited Vγ/Vδ gene usage such as Vγ1/Vδ6.
3) The γ/δ T cells are specialized to recognize 65 kd hsp but not heat-killed *Listeria*.
4) The early-appearing γ/δ T cells produce γIFN in response to 65 kd hsp.
5) The γ/δ T cell-depleted mice show impaired protection at the early stage after listerial infection.

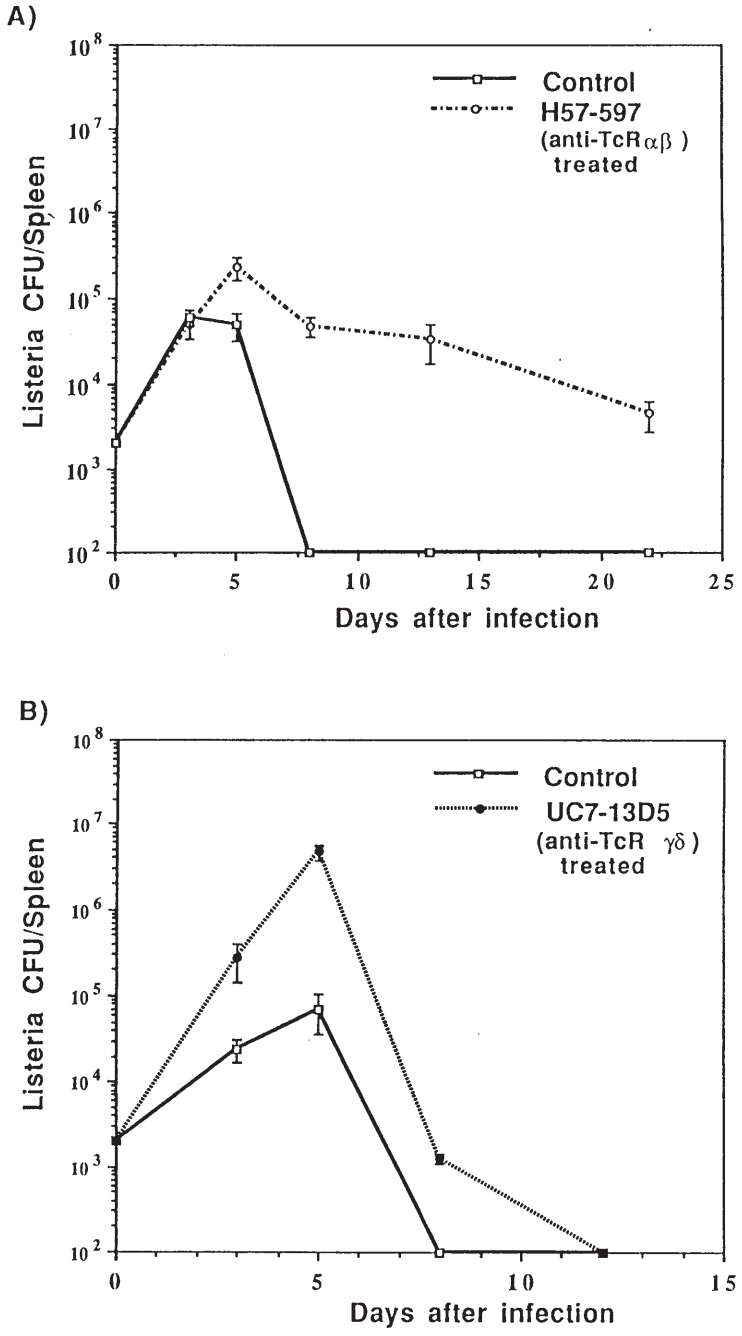


Fig. 4. Effects of *in vivo* administration of anti-TCR αβ (H57-597) (A) or anti-TCR γδ (B) on recovery of bacteria from spleens. Female C3H mice were inoculated intraperitoneally with 3×10^3 of *L.monocytogenes* on day 0 and with 200 μg of anti-TCR αβ mAb or 200 μg of anti-TCR γδ mAb on day 3. The number of *Listeria* recovered from spleens of infected mice on the indicated days were determined by colony formation assay on TSA. Values are means ± SD for a group of five mice.

Listeriosis is caused by the gram-positive rod, *L.monocytogenes*, which is one of the intracelluar bacteria as well as *Mycobacterium tuberculosis*. The protective mechanisms against listeriosis are largely divided into two phases.[15-17] The early response, which occurs during the first 48 h, is attributed to resident macrophages and early influx of bone marrow-derived phagocytes in the liver and spleen. The late response, beginning at about four days, is characterized by the proliferation of listerial antigen-dependent T cells which further enhance bacterial killing *in vivo*. The early-appearing γ/δ T cells may play a role in covering the gap between the phagocytic systme and the highly evolved type of immune responses mediated by α/β T cells in host defense against listerial infection.

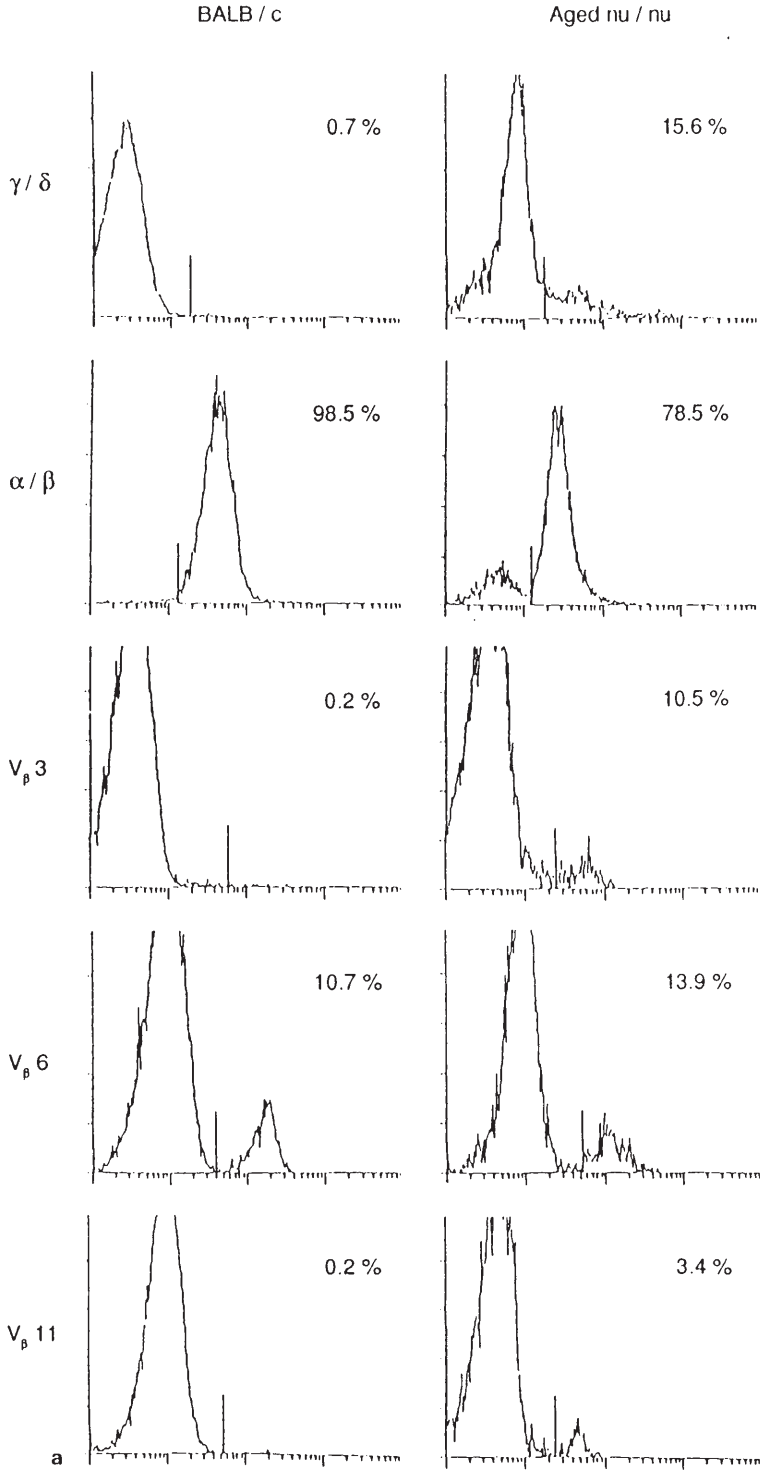
Hsp are polypeptides phylogenically highly conserved between eukaryotes and prokaryotes.[1,2] Under a variety of stress conditions such as heat shock, nutrient deprivation, and oxigen radicals, eukaryotic cells have been shown to produce stress proteins to preserve cellular functions.[5] Recently, Rajasekar et al. have reported that a subset of murine γ/δ T cells can react to antigens on self cells in which a heat-shock response is induced.[12] Born et al. have shown that the PPD-specific γ/δ T cell hybridomas derived from murine newborn thymus can respond to the equivalent portion of the autologous homolog to 65 kd mycobacterial hsp.[9,18] Thus, it is possible that the early-appearing γ/δ T cells during listeriosis may recognize endogenous hsp of autologous cells infected with *Listeria* as well as exogenous hsp derived from viable *Listeria*.

Our results with γ/δ T cells during listeriosis indicate that at least a subset of γ/δ T cells provides a first line of defense against infection, by recognizing 65 kd hsp and producing cytokines such as γIFN. Recently, we have found that the γ/δ T cells precede α/β T cells in appearance during infection with *Bacilllus Calmette Guerin* (BCG).[19] It may be generalized that γ/δ T cells may appear first in the infected site and the early-appearing γ/δ T cells may serve as a first line of defense against at least some infectious agents. Our finding is an important clue not only in elucidating the possible ligands for γ/δ T cells but also in understanding the functional role of γ/δ T cells in host defense mechanisms.

THE ROLE OF HSP-SPECIFIC γ/δ T CELLS AND α/β T CELLS IN INDUCTION OF AUTOIMMUNE DISEASES

Although T cells proliferate and differentiate primarily in the thymus, several studies with athymic nude mice and neonatally thymectomized (NTX) mice have revealed that an extrathymic pathway exists in T cell development.[20-25] Aged nude mice and NTX mice are reported to develop autoimmune diseases often.[26,27] To elucidate the mechanism of induction of the autoimune diseases in these mice, we have investigated the fate of self-reactive T cells and the responsiveness of T cells differentiating outside the thymus to 65 kd hsp.[28,29]

To confirm that T cells develop along an extrathymic pathway, we conducted FCM analysis for TcR/CD3 on LN cells of aged BALB/c nude mice and BALB/c NTX mice. An appreciable number of $CD3^+$ cells were detected in the LN cells of both aged nude mice and aged NTX mice (6 to 9 months old). Expression of TcR on the T cells in these mice was analyzed by double staining with anti-Thy1.2 mAb and anti-TcR α/β mAb or anti-TcR γ/δ mAb. As shown in Fig.5a, and appreciable nubmer of TcR γ/δ T cells were detected in the aged nude mice, while most of the T cells in the NTX mice expressed TcR α/β on their surface (Fig.5b). To examine the TcR repertoire of the TcR α/β T cells differentiating outside the thymus in nude mice and NTX mice, the LN cells were double stained with anti-Thy1.2 mAb and various anti-Vβ mAbs including anti-Vβ3, anti-Vβ6, anti-Vβ8 and anti-Vβ11 mAb. Consistent with


BALB / c
Aged nu / nu
γ / δ
0.7 %
15.6 %
α / β
98.5 %
78.5 %
Vβ 3
0.2 %
10.5 %
Vβ 6
10.7 %
13.9 %
Vβ 11
0.2 %
3.4 %
a
Fluorescence intensity

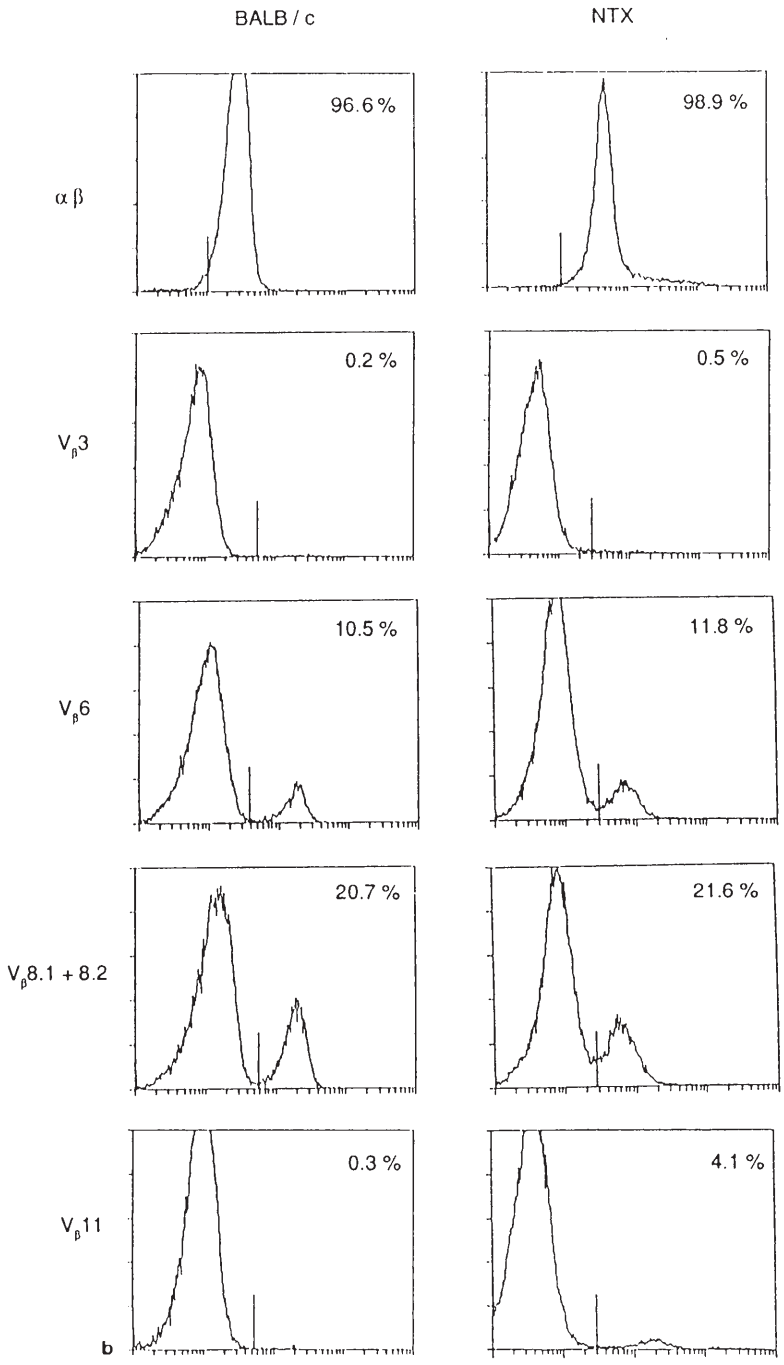


Fig. 5. Cell surface expression of TcR α/β, TcR γ/δ or Vβs on Thy1$^+$ cells in the LN cells from euthymic control BALB/c mice, aged nude mice (A) or NTX mice (B). The whole LN cell was stained with FITC-anti Vβ3, anti-Vβ6, anti-Vβ8.1+8.2, anti-Vβ11 mAb or anti-TCR αβ mAb, anti-TCR γδ mAb plus FITC-anti-hamster IgG and PE-anti-Thy1.2 mAb.

the earlier findings,[30,31] euthymic BALB/c control mice deleted Vβ3- or Vβ11-bearing T cells capable of recognizing Mls2[a] plus MHC class 2IA/IE, or MTV(mammary tumor virus)-related antigens plus IE, respectively, in a mature T cell pool as a result of intrathymic clonal deletion.[32,33] On the other hand, an appreciable number of Vβ11-bearing T cells were present in the LN of both the nude mice and NTX mice. Although the Vβ3-bearing T cells were eliminated in the LN of NTX mice, Vβ3-bearing T cells were present in the LN of nude mice (Fig.5a,b). These results suggest that T cells differentiating along an extrathymic pathway have not undergone negative selection and that timing of clonal deletion may be different in each self-reactive T cell population.[34]

To investigate the functional aspect of the self-reactive T cells in nude mice and NTX mice, we tried to activate the Vβ3- or Vβ11-bearing T cells by SEA, a superantigen that specifically stimulates T cells bearing Vβ3 or Vβ11 irrespective of the α chain expressed by these T cells.[35] Vβ3-and Vβ11-bearing T cells in the nude mice and Vβ11-bearing T cells in the NTX mice proliferated significantly in response to SEA in the presence of exogenous IL-2 (data not shown). No Vβ3-bearing T cells proliferated in response to SEA because of tolerance-induced deletion of self-reactive T cells. Thus, the self-reactive T cells in the nude mice and NTX mice can normally respond to stimulation via their TcR and be functional.

It is well known that NTX mice develop multiple organ-localized autoimmune diseases.[27] Nude mice are also known often to develop autoimmune diseases.[27] To confirm whether aged nude mice and NTX mice spontaneously develop autoimmune diseases, we investigated the

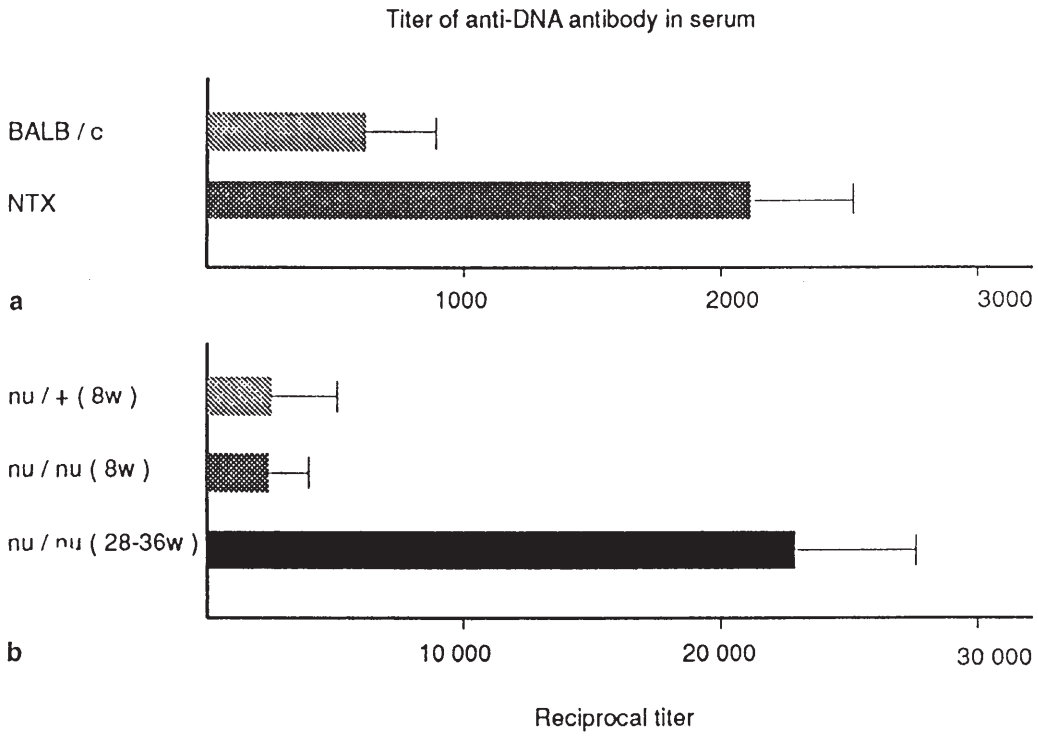


Fig. 6. Titers of anti-DNA antibody in the serum of BALB/c control mice, NTX mice (A) or aged nude mice (B). Anti-DNA titers in the serum were measured by ELISA. Each column and horizontal bar represent the mean of three individual mice ± SD.

level of anti-DNA antibody in the serum and the stomach histology of these mice. As shown in Fig.6a, anti-DNA titers in sera of NTX mice were much higher than those in the control euthymic mice. Histological examination revealed that the NTX mice had gastritis characterized by infiltration of many mononuclear cells into the mucosa and submucosa of the stomach (data not shown). Although aged nude mice bred under SPF condition did not develop autoimmune disease, a substantially higher titer of anti-DNA antibody was detected in the sera of the aged nude mice bred under conventional conditions (Fig.6b).

To determine whether T cells capable of recognizing 65 kd hsp are involved in teh development of autoimmune diseases in aged nude mice and NTX mice, we tested the reactivity of LN cells to recombinant 65 kd hsp derived from *Mycobacterium bovis* in aged nude mice and NTX mice. The LN cells of the nude mice and NTX mice proliferated notably and produced a higher level of IL-2 in response to the 65 kd hsp as compared with those in normal mice (Fig.7a,b). These results suggest that aged nude mice and NTX mice may contain an increased number of 65 kd hsp-specific T cells in their mature T cell pool. To investigate which population of T cells proliferate in response to 65 kd hsp in these mice, we examined by FCM the responder cells after culture with 65 kd hsp. As shown in Fig.8a, the proportion of γ/δ T cells in aged nude mice bred under conventional conditions substantially increased after stimulation with PPD, recombinant 65 kd hsp, or trypsin-treated hsp. PCR analysis with Vγ and Vδ primers revealed that the 65 kd hsp-specific γ/δ T cells preferentially expressed Vγ1/Vδ6 (data not shown). In case of NTX mice, hsp-responding cells were mainly TcR α/β T cells rather than TcR γ/δ T cells. Among TcR α/β T cells, Vβ11-bearing T cells proliferated greatly after culture with the 65 kd hsp, whereas the proportions of other T cells bearing Vβ6 or Vβ8.1+8.2 did not increase (Fig.8b). To further determine whether Vβ11-bearing T cells enriched after culture with the 65

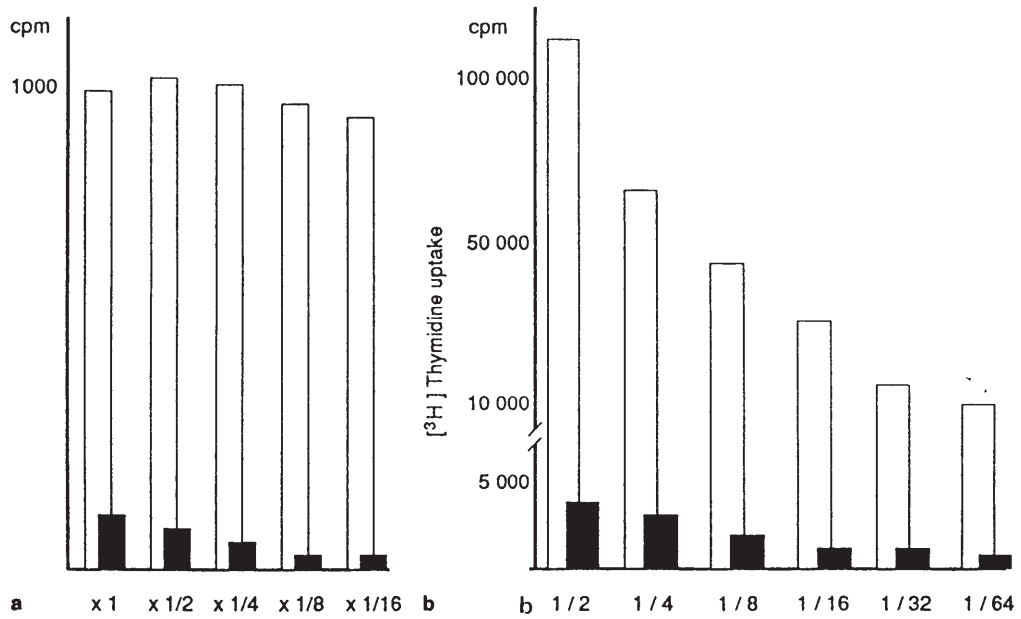


Fig. 7. IL-2 production of the LN cells from aged nude mice (A) or NTX mice (B) stimulated with 65 kd hsp. CTLL-2 cells (2×10^4) were incubated with diluted supernatants from the cultures in which LN cells were stimulated with 65 kd hsp (25 μg/ml) in 0.2 ml medium at 37C for 48 h, and then pulsed with 1μCi [^{3}H]-dThd. Cultures were incubated for another 6 h and cells were harvested.

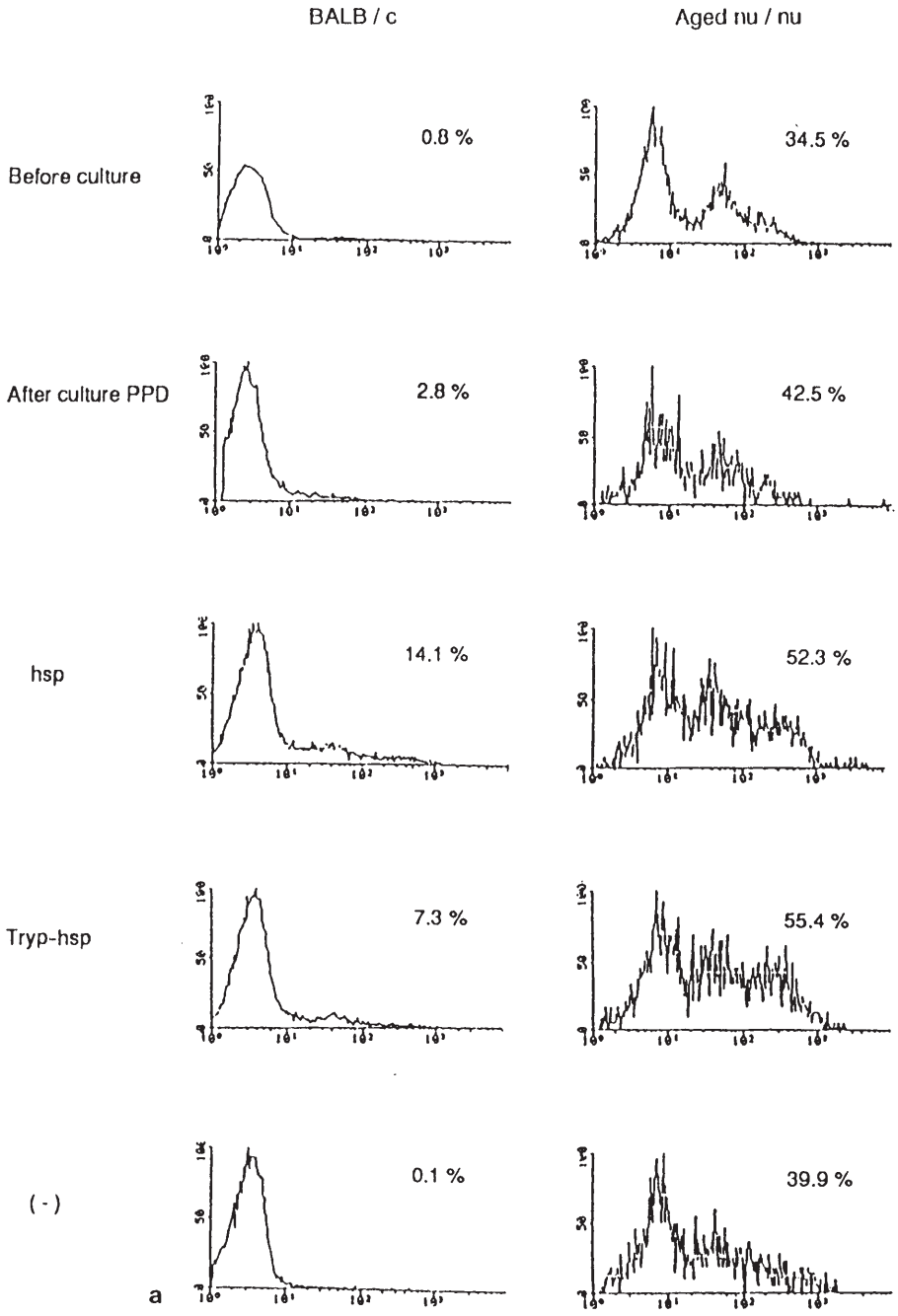


Fig. 8. (A) Responsiveness of normal BALB/c (a) or aged nude mice (b) γ/δ T cells to PPD or mycobacterial hsp. LN cells from aged nude mice were cultured with PPD (30 μg/ml), recombinant 65 kd of *M.bovis* (25μg/ml) or trypsin-digested (Try-hsp) hsp (25μg/ml) for 3 days. Cultured cells were stained with anti-TcR γ/δ mAb and with anti-Thy-1.2. Thy-1.2$^-$ cells were gated out and shown are histograms of TcR γ/δ expression of Thy-1.2$^+$ cells.

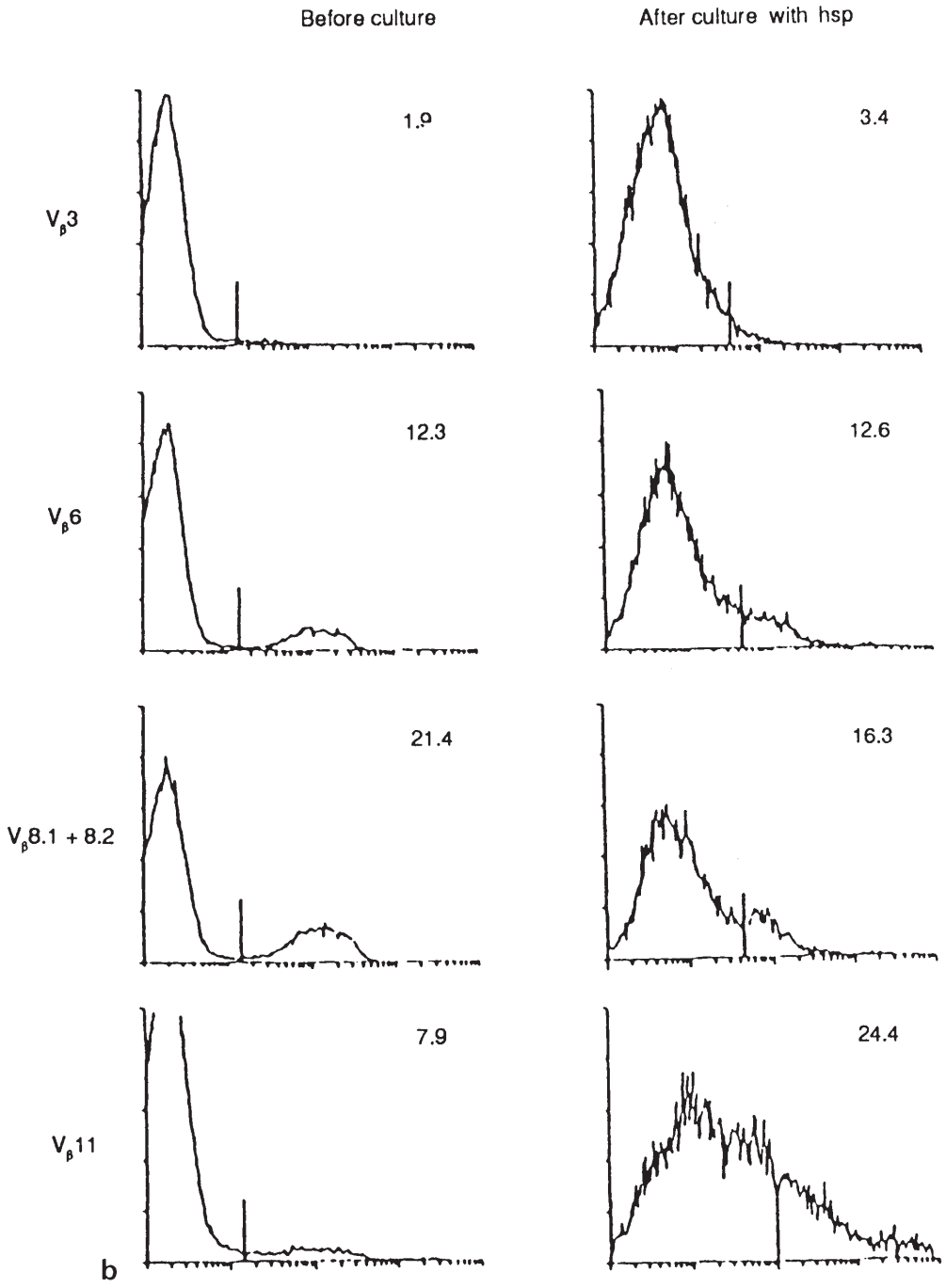


Fig. 8. (B) Activation of Vβ11-bearing T cells in NTX mice by 65 kd hsp. The LN cells (1×10^6/ml) from NTX mice were cultured with the 65 kd hsp at 25 μg/ml for three days. Cultured cells were stained with anti-Vβ3 mAb, anti-Vβ6 mAb, anti-Vβ8.1+8.2 mAb, or anti-Vβ11 mAb plus FITC-anti-hamster or anti-rat IgG and PE-anti-Thy-1.2 mAb, FITC-anti-CD8 mAb, PE-anti-CD4 mAb.

kd hsp were really in an activated state, the size of the Vβ11-bearing T cells was assessed by their forward light scatter profile. Approximately half of the Vβ11-bearing T cells were blastoid cells, whereas Vβ8.1+8.2-bearing T cells were small. (data not shown). Thus, Vβ11-bearing T cells may proliferate substantially after culture with 65 kd hsp.

A notable finding in the present sutdy was that the aged nude mice and NTX mice that developed autoimmune diseases contained a large number of 65 kd hsp-specific T cells in their peripheral lymphoid tissues. In aged nude mice, the hsp-responding T cells are mainly Vγ1/Vδ6-bearing T cells, whereas self-reactive Vβ11-bearing T cells in NTX mice increased in number after culture with the 65 kd hsp. Both T cells seem to produce a high level of IL-2 in response to 65 kd hsp. Hsp are polypeptides phylogenetically conserved between prokaryotes and eukaryotes.[2] We speculate that the 65 kd hsp-specific T cells may be activated by hsp expressed by bacteria and autologous cells stressed with various pathogens, resulting in the development of autoimmune diseases in such animals.

Several lines of evidence have shown that tolerance to self-antigens is mainly due to intrathymic clonal deletion of self-reactive T cells.[30,31] Our results reveal that a substantial number of self-reactive T cells such as Vβ11-bearing T cells were present in the LN of nude mice and NTX mice. These self-reactive T cells can respond normally to signals via the TcR in the presence of exogenous IL-2. Our results also revealed that the self-reactive Vβ11-T cells in NTX mice proliferate substantially after culture with 65 kd hsp. These T cells expressed a high level of IL-2Rα even before stimulation.[28,29] Therefore it is possible that hsp-reactive T cells other than Vβ11-bearing T cells may respond to the 65 kd hsp and subsequently produce a stimulus such as IL-2 for proliferation of the self-reactive Vβ11-bearing T cells. In case of aged nude mice, hsp-responding cells are found mainly in γ/δ T cells expressing Vγ1/Vδ6. At present, we can not detect any increase in the number of particular T cells including γ/δ T cells other than Vβ11-bearing T cells in the NTX mice. Recently we have found that an autoreactive T cell line specific for self MHC class II recognized 65 kg hsp.[36] Therefore, it is alternatively possible that Vβ11-bearing T cells in NTX mice can directly recognize the 65 kd hsp in the context of a self MHC-molecule. The reactivity of Vβ11-bearing T cells to the 65 kd hsp should be determined at a clonal level to clarity this possibility.

The self-reactive T cells are rendered tolerant mainly by clonal energy and clonal suppression via suppressor cells.[37,38] although recent studies reveal that clonal deletion occurs extrathymically.[39-41] It has been reported that autoimmune diseases in NTX mice were clearly prevented by the reconstitution of these mice with T cells from syngeneic, normal animals. A deficit of suppressor T cells may be a cause of autoimmune disease in NTX mice.[42] The high reactivity of T cells to the 65 kd hsp in NTX mice may result from depletion of a specific T cell subpopulation responsible for checking and controlling the hsp-specific T cells which is normally presnet in the mature T cell pool in euthymic mice. An increase in the number of 65 kd hsp-specific T cells was evident in athymic nude mice bred under conventional conditions. Environmental antigens such as those of intestinal microflora may also play an important role in the expansion of the hsp-specific T cells in aged nude and NTX mice.

CONCLUDING REMARKS

Our results presented here suggest that 65 kd hsp-specific T cells play an importnat role not only in induction of autoimmune diseases but also in the host defense mechanism against infection by various pathogens. During infection with *L.monocytogenes*, TcR γ/δ T cells specific for 65 kd hsp precede TcR α/β T cells specific for the listerial antigen in appearance. The γ/δ T

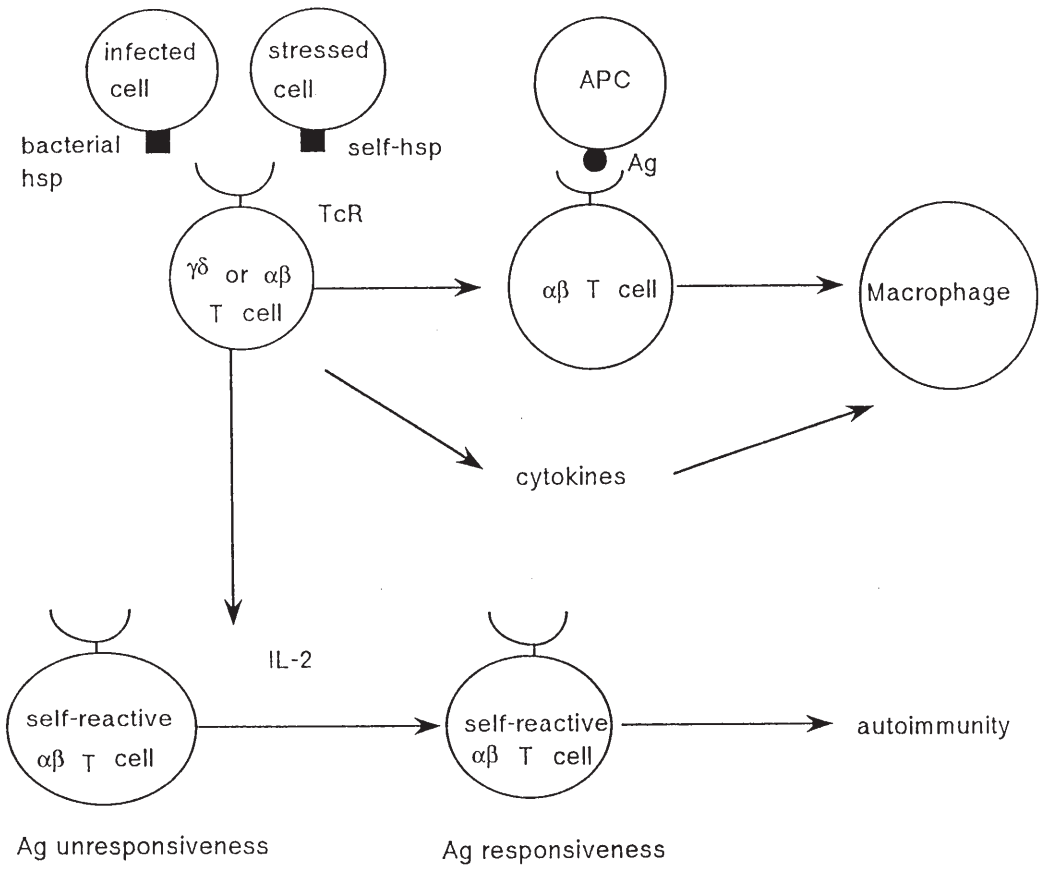


Fig. 9. Model of roles of hsp-specific T cells in host defense against bacteiral infection and induction of autoimmune disease.

cells seem to provide a first line of defense against the infection by recognizing exogenous and endogenous 65 kd hsp on infected cells and producing cytokines such as γIFN. Thus, the hsp-specfic T cells may respond quickly to antigenically diverse pathogens before antigen-specific T cells expand clonally, and they may play a role in covering the gap between the phagocytic system and the highly evolved type of immune responses (Fig.9).[43,44]

Athymic mice such as nude mice and NTX mice contain an appreciable number of self-reactive T cells that have not undergone inrathymic clonal deletion. The self-reactive T cells express IL2 receptor and respond normally to signals delivered through the TcR in the presence of exogenous IL2. Notably, a substantially higher level of hsp reactivity was observed in the LN cells in such animals, especially those bred under conventional conditions. In aged nude mice, γ/δ T cells greatly proliferated and produced IL2 in response to 65 kd hsp. On the other hand, the self-reactive Vβ11-bearing T cells notably proliferated and a high level of IL-2 was detected after culture with 65 kd hsp in the NTX mice. Both groups of animals spontaneously developed autoimmune diseases. Taking all the previous results into consideration, we speculate that 65 kd hsp-specific T cells may abrogate the unresponsiveness of the self-reactive T cells and/or B cells by producting IL-2 and that they may play an important role in the induction to autoimmune diseases (Fig.9).

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