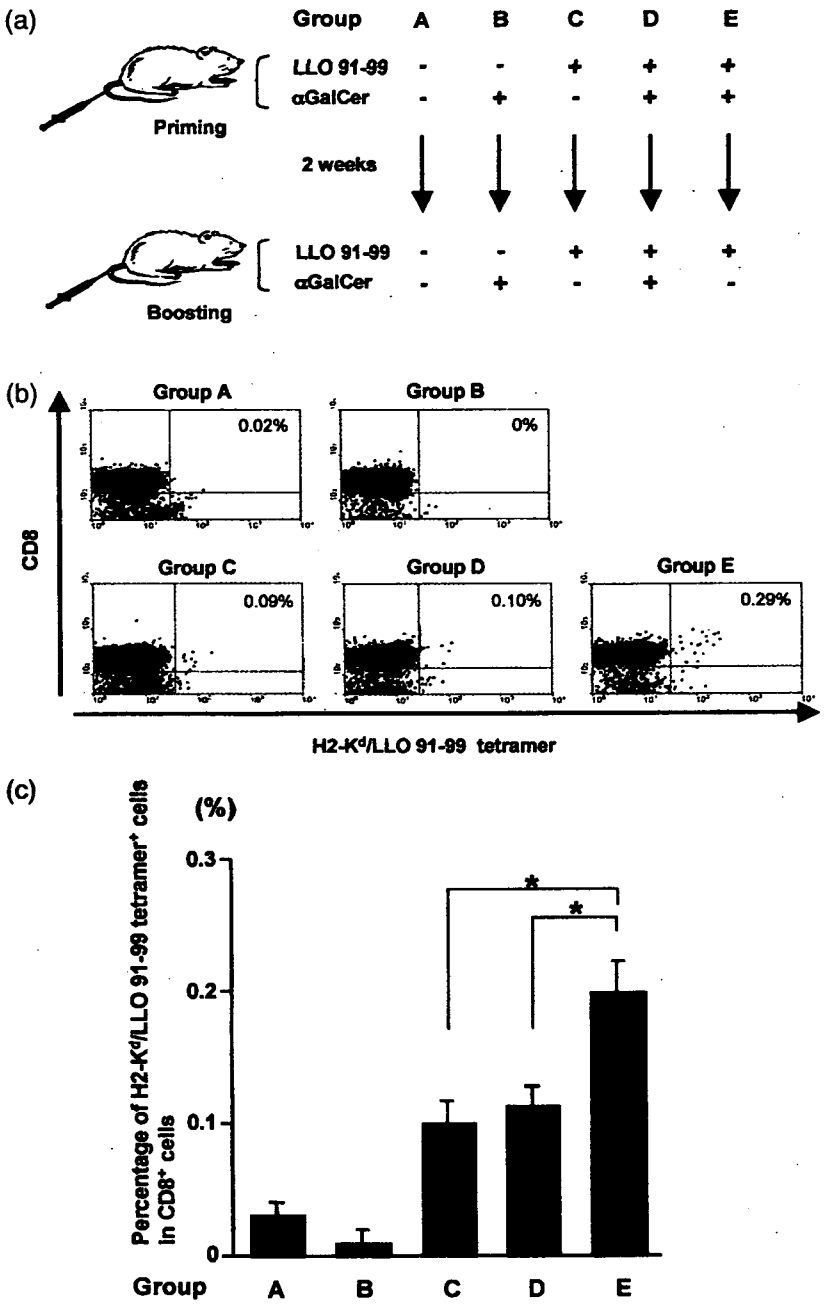

Fig. 1. Induction of LLO91-99-specific CD8$^+$ T cells in the spleens of LLO91-99 peptide and/or αGalCer-pulsed DC-immune mice. (a) Immunization schedule of mice with DCs pulsed with LLO91-99 peptide and/or αGalCer. Mice were immunized with DCs pulsed with LLO91-99 peptide and/or αGalCer twice with a 2 week interval. Group A, mice given untreated DCs twice; group B, mice given DCs pulsed with αGalCer twice; group C, mice given DCs pulsed with LLO91-99 peptide alone twice; group D, mice given DCs pulsed with both LLO91-99 peptide and αGalCer twice; group E, mice given DCs pulsed with both LLO91-99 peptide and αGalCer at priming phase and DCs pulsed with LLO91-99 peptide alone at boosting phase. (b) Flow cytometric analysis for LLO91-99-specific CD8$^+$ T cells after boost DC immunization. H2-K^d/LLO91-99 tetramer staining was performed 3 days after boost immunization with LLO91-99 peptide and/or αGalCer-pulsed DCs. The percentages of the tetramer$^+$ cells in CD8$^+$ T cells of each experimental group are shown. (c) Summary of the percentages of H2-K^d/LLO91-99 tetramer$^+$ cells in CD8$^+$ T cells of each experimental group. The means ± SE of five mice per group are shown. Asterisks indicate significant difference ($P < 0.01$).

Quantitation of IFN-γ in splenocyte culture supernatants by sandwich ELISA

Pools of spleen cell suspensions (2×10^6 cells mL^{-1}) from groups of mice immunized with DCs were cultured in RPMI/10FCS in 24-well plates in the presence or absence of 1 μM LLO91-99 peptide at 37 °C in 5% CO_2 atmosphere. Supernatants were harvested after 3 days and stored at −20 °C until assayed. The concentration of interferon-γ (IFN-γ) in the culture supernatants was determined using sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine®; R&D Systems).

Quantitation of serum cytokines using a cytometric beads array

Blood was obtained from the lateral tail veins of mice, before and 6, 12, 24 and 36 h after the prime and boost immunizations. The concentrations of cytokines [IFN-γ, interleukin (IL)-12p70, IL-4, IL-10, tumor necrosis factor-α (TNF-α) and IL-6] in each serum were evaluated with a BD mouse inflammation cytometric bead array kit and a BD Th1/Th2 cytokine cytometric bead array kit (BD Biosciences PharMingen) according to the manufacturer's instructions.

Monitoring CTL memory cells

Four weeks after the boost immunization, splenocytes from immunized mice were stained with the phycoerythrin-conjugated tetrameric H2-K^d/LLO91-99 peptide complex and phycoerythrin-Cy5-conjugated antimouse CD8α monoclonal antibody (mAb) (BD Biosciences PharMingen). Simultaneously, these cells were stained with FITC-conjugated antimouse CD44 mAb (eBioscience, San Diego, CA) or FITC-conjugated antimouse CD62L mAb (eBioscience), and analyzed using an EPICS XL digital flow cytometer (Beckman Coulter).

In vivo CTL assay

The *in vivo* CTL assay was as originally reported by Aichele *et al.* (1997). Spleens from naïve BALB/c mice were removed aseptically and homogenized. Erythrocytes were depleted from the spleen cell suspension by lysis with 0.83 M NH_4Cl solution, washed in RPMI 1640, resuspended at 2×10^7 cells mL^{-1} in RPMI/10FCS, and incubated for 1 h at 37 °C in 5% CO_2 atmosphere in the presence or absence of 1 μM LLO91-99 peptide. After the incubation, the cells were washed, resuspended in PBS at 2×10^7 cells mL^{-1}, and labeled for 5 min at 37 °C in the dark with 5 (6)-carboxyfluorescein diacetate succinimidyl ester (CFSE; Dojindo, Kumamoto, Japan) either at 0.5 μM (unpulsed cells; $CFSE^{lo}$) or 5 μM (peptide-pulsed cells; $CFSE^{hi}$). Cells were washed with PBS supplemented with 5% FCS and resuspended in PBS at 5×10^7 cells mL^{-1}. Ten million cells of a 1 : 1 mixture of $CFSE^{lo}$: $CFSE^{hi}$ were adoptively transferred into immune BALB/c mice. Eighteen hours later, the adoptively transferred mice were sacrificed, the spleens were removed and homogenized, the erythrocytes were depleted, and the cells were washed and resuspended in PBS. The cells were then analyzed with an EPICS XL digital flow cytometer (Beckman Coulter). To evaluate the percentage of specific lysis, the ratio of $CFSE^{lo}$: $CFSE^{hi}$ in immune mice was compared with that in the transferred naïve control mice. For each experimental group, at least three animals were tested.

Bacterial infection and evaluation of antilisterial immunity

Four weeks after boosting immunization, the immunized mice were challenged with 5×10^4 CFU (*c.* 5 LD_{50}) of *L. monocytogenes* i.v. Seventy-two hours after the challenge infection, bacterial numbers in the spleens were determined by plating 10-fold dilutions of the tissue homogenates on trypticase soy agar plates.

Blocking boost immunization with IFN-γ neutralizing mAb

Two days before boost immunization, 3 mg per mouse of IFN-γ neutralizing mAb, which was obtained from R4-6A2 cells (ATCC) culture supernatant, were injected i.p. Subsequently, we monitored the $CD8^+$ T-cell proliferation with MHC tetramer 3 days after the boost immunization, and the $CD8^+$ memory T cells and antilisterial immunity 4 weeks after boost immunization.

Statistical analysis

Data from multiple experiments were expressed as the mean ± SE. Data were analyzed by Fisher's protected least significant difference (PLSD). Statistical analysis was performed using STATVIEW-J 4.5 (SAS Institute Inc., Cary, NC). All tests were two-sided and performed at the 0.05 significance level.

Results

Immunization with LLO91-99 peptide-loaded DC pulsed with αGalCer at priming phase, but not at boosting phase, augmented specific $CD8^+$ T-cell responses

In order to see the effects of αGalCer-pulsed DCs on specific $CD8^+$ T-cell induction *in vivo*, we first evaluated LLO91-99 peptide-specific $CD8^+$ T-cell induction in the spleen cells of mice immunized with DCs pulsed with LLO91-99 peptide alone, or with LLO91-99 peptide and αGalCer by H2-K^d/LLO91-99 tetramer analysis. We immunized BALB/c mice twice (at priming and boosting phases) with a 2 week

interval with syngeneic BM-DCs pulsed with LLO91-99 peptide and/or αGalCer, as shown in Fig. 1(a).

First, we examined the specific $CD8^+$ T-cell induction 3 days after boosting immunization (Fig. 1b and c). As expected, LLO91-99 peptide-pulsed (groups C to E), but not unpulsed (groups A and B), DC immunization induced H2-K^d/LLO91-99 $tetramer^+$ $CD8^+$ T cells. The spleen cells of group E mice showed a significantly higher percentage of $tetramer^+$ cells in the whole $CD8^+$ T-cell population when compared with those of group C mice ($P = 0.0019$) or group D mice ($P = 0.0042$). These percentages of $tetramer^+$ cells in the $CD8^+$ T-cell population correlated well with the absolute numbers of $tetramer^+$ cells in the $CD8^+$ T-cell population (data not shown).

Immunization with DCs pulsed with αGalCer induced large amounts of IFN-γ production from immune spleen cells

Free αGalCer administration has been reported to induce large amounts of IFN-γ *in vivo* (Fujii *et al.*, 2002). In order to see the effects of immunization of DCs pulsed with αGalCer on IFN-γ production, we examined IFN-γ production by immune spleen cells after the immunization. Three days after the boost injection of DCs, spleen cells from the immunized mice were obtained and cultured with or without LLO91-99 peptide for 3 days. Supernatant of each sample was harvested after 3 days of culture, and the amounts of IFN-γ in it were determined by sandwich ELISA. Immunization with unpulsed DCs alone induced high amounts of IFN-γ from the immune spleen cells (Fig. 2).

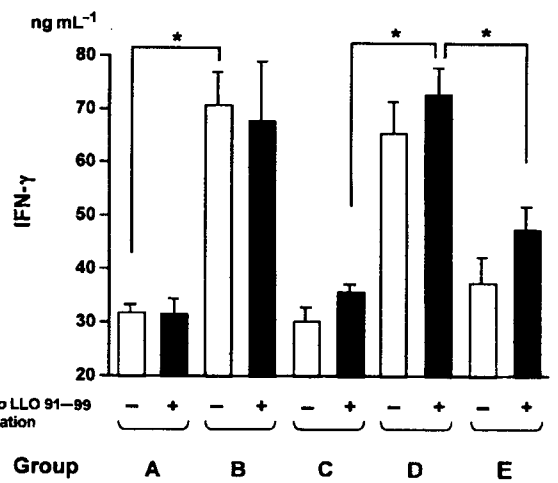

Fig. 2. Immunization with αGalCer-pulsed DCs induced large amounts of IFN-γ production by the immune spleen cells. The immune spleen cells were removed 3 days after boost immunization and the cells were cultured with or without LLO91-99 peptide. The supernatants were harvested after 3 days of culture and the concentration of IFN-γ of the supernatants was determined by sandwich ELISA. The means ± SE of five mice per group are shown. The asterisks indicate statistical significance ($P < 0.01$).

Immunization with αGalCer-pulsed DCs induced more than two times higher amounts of IFN-γ compared with the amounts induced by immunization with untreated DCs (Fig. 2: A(–) and B(–), $P = 0.0001$). When LLO91-99 peptide was also pulsed on DCs, LLO91-99 peptide stimulation *in vitro* increased the amounts of IFN-γ especially in group E, but the level of increase was minimal compared with the baseline level, especially in group D. Therefore, large amounts of IFN-γ produced by immune spleen cells were mainly attributable to αGalCer pulsed on DCs. We tried to observe induction of NKT cells by αGalCer-pulsed DC injection, but observed a rapid decrease of NKT cell number after the injection (data not shown). This would appear to be due to down-modulation of T-cell receptor and NK1.1 molecule on NKT cells rather than activation-induced cell death (Crowe *et al.*, 2003; Wilson *et al.*, 2003; Harada *et al.*, 2004).

Immunization with DCs pulsed with αGalCer at priming phase significantly reduced the bacterial load in the spleens of LLO91-99 peptide-pulsed DC immunized mice after *Listeria* challenge

We next examined whether αGalCer treatment of LLO91-99 peptide-pulsed DC affects the antilisterial protective immunity by the DC immunization. We immunized BALB/c mice with DCs loaded with LLO91-99 peptide and/or αGalCer at priming and boosting phases. Four weeks after the boost immunization, the immune mice were challenged with a lethal dose of *L. monocytogenes*. Three days later, bacterial CFUs in the spleens were evaluated. As shown in Fig. 3, group A and B mice did not show a significant decrease in CFU compared with naïve mice. The group C mice showed a significant decrease in CFU ($P = 0.0158$) to a level equivalent to the CFU in mice given LLO91-99 minigene-DNA vaccine (p91mam plasmid) three times. Group D mice tended to show a lower number of CFU compared with group C mice, but not significantly so ($P = 0.309$). Surprisingly, the group E mice showed the lowest number of CFU among the DC-immunized mice groups, and the difference in the number of CFU between groups C and E was significant ($P = 0.0379$).

Immunization with DCs pulsed with αGalCer at priming phase significantly enhanced the specific CTL activity by LLO91-99 peptide-pulsed DC immunization *in vivo*

We then analysed the specific CTL activity *in vivo* in these immune mice 4 weeks after the last immunization. As shown in Fig. 4, the group E mice showed the highest LLO91-99 peptide-specific CTL activity *in vivo* among the DC-immunized mice groups (groups C and E, $P = 0.029$; groups D and E, $P = 0.0005$; LLO91-99 DNA and group E,

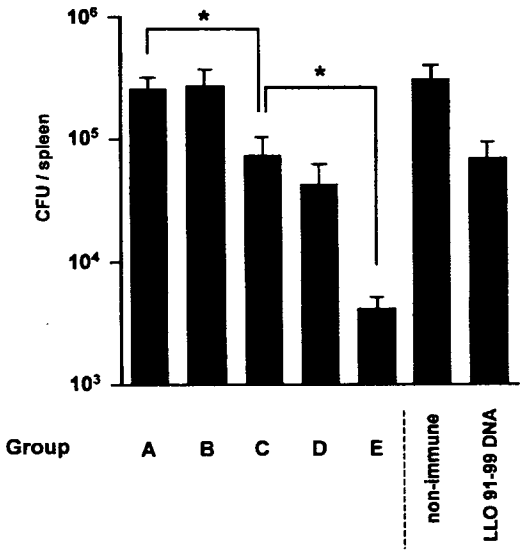

Fig. 3. *In vivo* protection of DC-immune mice against lethal *L. monocytogenes* challenge infection. Four weeks after the last immunization, the mice were challenged with 5×10^4 CFU of *L. monocytogenes* i.v. The antilisterial protective immunity induced by the DC immunization was evaluated by counting the bacterial numbers in the spleens 72 h after challenge infections by plating 10-fold dilutions of tissue homogenates. The means ± SE of eight mice per group are shown. The asterisks indicate statistical significance ($P < 0.05$).

$P < 0.0001$), although the *in vivo* CTL activity in the mice immunized with a sublethal dose of *L. monocytogenes* was much higher. This result suggests that the specific CTL activity *in vivo* reflects the protective immunity against lethal listerial challenge.

Immunization with DCs pulsed with αGalCer at the priming phase alone augmented the specific central memory CD8$^+$ T-cell number produced by LLO91-99 peptide-pulsed DC immunization

The induction of memory T cells is the most critical issue in vaccination against intracellular pathogens. Memory T cells have been divided into two major subtypes, effector and central memory T cells, which are characterized with effector memory (CD44$^+$ CD62L$^-$) and central memory (CD44$^+$ CD62L$^+$) phenotypes, respectively (Lefrançois & Masopust, 2002), and the latter has been reported to be critical for effective responses to secondary pathogen challenge (Wherry *et al.*, 2003; Zaph *et al.*, 2004; Roberts *et al.*, 2005; Vaccari *et al.*, 2005). Therefore, we examined the effect of αGalCer-pulsed DC immunization on the induction of specific memory CD8$^+$ T cells. Four weeks after boost immunization, immune spleen cells were prepared, and CD44 and CD62L (memory T-cell markers) expression on the H2-K^d/LLO91-99 tetramer$^+$ CD8$^+$ T-cell subset was examined. The percentage of CD44$^+$ cells in H2-K^d/LLO91-99 tetramer$^+$ CD8$^+$ T cells was significantly higher in the group E mice than the group C mice ($P = 0.0381$, Fig. 5a). No difference between group D mice and group E mice was observed in the percentage of CD44$^+$ cells in H2-K^d/LLO91-99 tetramer$^+$ CD8$^+$ T cells (Fig. 5a). However, the percentage of CD62L$^+$ cells in H2-K^d/LLO91-99 tetramer$^+$ CD8$^+$ T cells was significantly higher in the group E mice compared with those in the group D ($P = 0.0092$) mice and LLO91-99 DNA (p91mam)-immunized mice ($P = 0.0098$) (Fig. 5b). This result suggests that the percentage of central memory cells in total memory CD8$^+$ T cells in group E was higher than that in group D, although the percentage of total memory cells in LLO91-99-specific CD8$^+$ T cells was similar in groups D and E.

Rapid induction of various cytokines in sera of mice immunized with DCs pulsed with αGalCer

We were curious about why group E mice induced significant levels of specific CTL activity *in vivo* and the protective immunity. Cytokine milieu in the induction of *in vivo* CTL may affect the nature of CTL. We therefore examined several cytokine levels in sera of immune mice immediately after the first and second DC injections (Fig. 6). At priming phase, a rapid increase of the serum IFN-γ level was observed in group D or E mice compared with group C mice at 12 h after DC injection ($P = 0.0316$). At boosting phase, much greater amounts of Th1-type cytokines (IFN-γ, IL-12p70) were detected in the sera of group D mice compared with group C and E mice at 6 h after injection ($P < 0.0001$ respectively). Serum IFN-γ level in group D mice was almost 100-fold greater than that in group C and E mice at boosting phase. In addition, the serum levels of Th2-type cytokine (IL-4) as well as inflammatory cytokines (TNF-α, IL-6) were also significantly enhanced in group D mice compared with group C and E mice at boosting phase ($P < 0.0001$ respectively). Intriguingly, group E mice showed low levels of these cytokines, as well as IFN-γ and IL-12p70, at boosting phase.

Blockade of IFN-γ at boosting phase retrieved the induction of LLO91-99-specific CD8$^+$ central memory T cells and the reduction of the bacterial load in the spleens of *Listeria*-challenged mice

Among the variety of cytokines produced in sera after boost DC injection, we speculated that IFN-γ was the key cytokine influencing the subsequent T-cell responses because IFN-γ amounts were saliently produced in sera at boosting phase in group D, which showed the attenuated protective immunity. We were therefore interested in the effects of IFN-γ burst at boosting phase in the subsequent immune responses. To clarify the effects, we injected IFN-γ-neutralizing mAb 2 days before boost DC immunization to block the

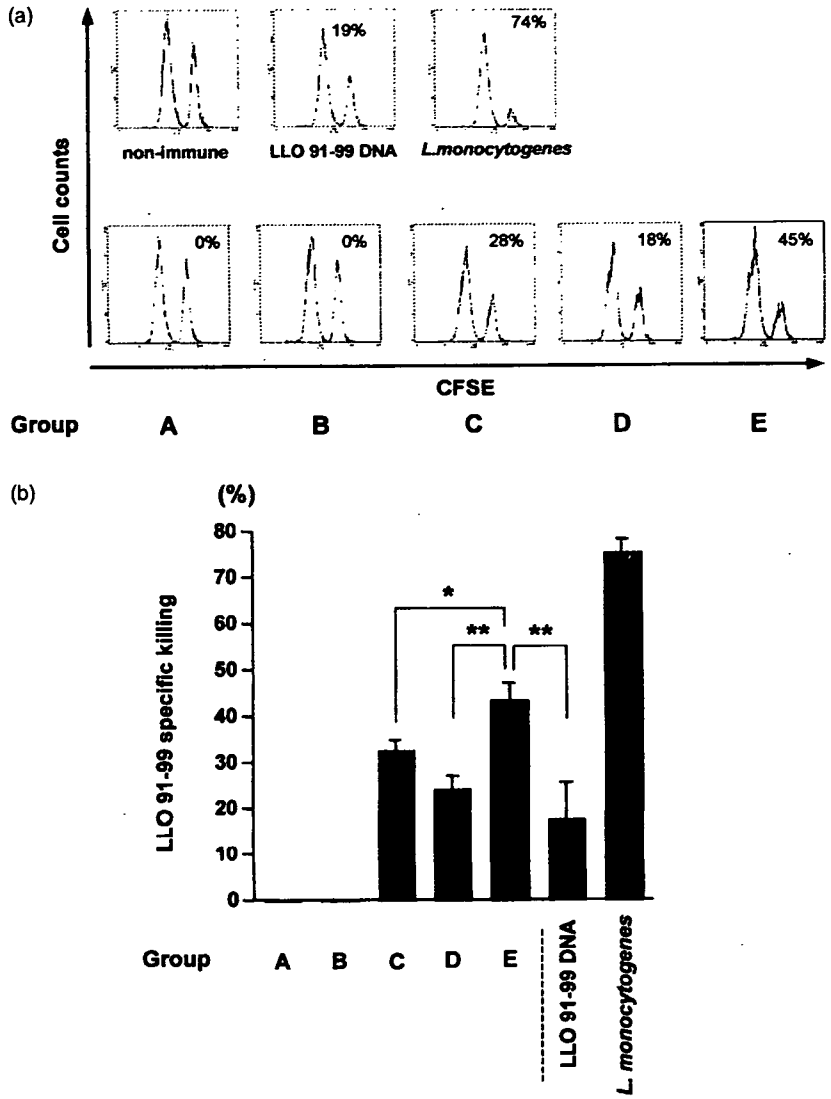

Fig. 4. *In vivo* CTL activity specific for LLO91-99 peptide-pulsed spleen cells. (a) Flow cytometric analysis of *in vivo* CTL activity specific for LLO91-99-pulsed spleen cells. Four weeks after boost immunization, LLO91-99 peptide and/or αGalCer-pulsed DC-immune mice were adoptively transferred with a 1 : 1 mixture of unpulsed $CFSE^{lo}$-labeled and the peptide-pulsed $CFSE^{hi}$-labeled naïve spleen cells 18 h before the analysis. The percentage of lysis for each experimental group is shown. The data of LLO91-99-expressing plasmid (p91mam) DNA-immune, or a sublethal dose (10^3 CFU) of *Listeria*-immune mice are also shown as controls. (b) Summary of the percentage of lysis of each experimental group obtained with *in vivo* CTL assay. The means ± SE of six mice per group are shown. Single asterisk and double asterisks indicate significant differences of $P < 0.05$ and $P < 0.01$, respectively.

IFN-γ burst at boosting phase, and examined the effects on the subsequent immune responses. We observed an increased number of LLO91-99-specific $CD8^+$ T cells in the mAb-treated group D mice compared with the mAb-untreated mice group (Fig. 7a). Furthermore, the number of $CD62L^+$ central memory $CD8^+$ T cells specific for LLO91-99 peptide in the mAb-treated group D mice was significantly increased ($P = 0.0158$), and was comparable with the number of these cells in group E mice (Fig. 7b). In parallel, the bacterial load in the spleens of *Listeria*-challenged mice 4 weeks after the boost immunization was improved in the mAb-treated group D mice ($P = 0.0380$, Fig. 7c). Administration of neutralizing mAb against IFN-γ in boosting phase did not affect the protective immunity in group E mice. Taken together, these results suggest that IFN-γ burst at boosting phase is involved in the exacerbating effect on the establishment of the effective specific $CD8^+$ memory T-cell pool.

Discussion

As DCs are the most powerful APCs that can initiate primary immune responses, DC vaccination has been an

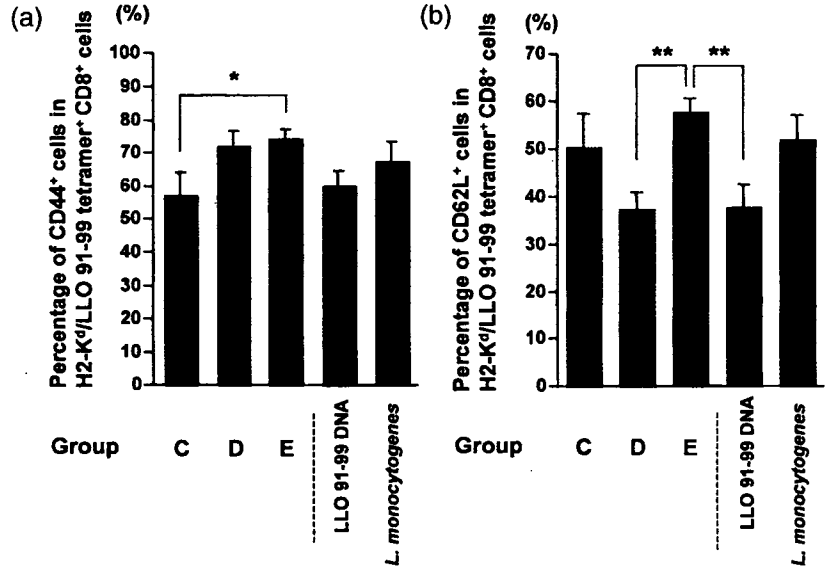

Fig. 5. Expression of CD44 and CD62L on LLO91-99-specific $CD8^+$ T cells in spleens of LLO91-99 peptide and/or αGalCer-pulsed DC-immune mice. (a) Summary of the percentage of $CD44^+$ cells in the H2-K^d/LLO91-99 $tetramer^+$ $CD8^+$ T cells. Flow cytometric analysis for H2-K^d/LLO91-99 tetramer and CD44 staining was performed 4 weeks after boost immunization. The means ± SE of five mice per group are shown. A single asterisk indicates a significant difference ($P < 0.05$). (b) Summary of the percentage of $CD62L^+$ cells in the H2-K^d/LLO91-99 tetramer-positive $CD8^+$ T cells. The immune spleen cells were analyzed as in (a) using FITC-conjugated antimouse CD62L mAb. The means ± SE of five mice per group are shown. Double asterisks indicate a significant difference ($P < 0.01$).

attractive strategy for combating tumors and infectious diseases (Mayordomo *et al.*, 1995; Celluzzi *et al.*, 1996). Several reports have tried to take advantage of αGalCer administration in order to strengthen the immunization effects of DC vaccination. αGalCer strongly activates *i*NKT cells. *i*NKT cells interact with DCs through CD40–CD40 ligand interaction (Fujii *et al.*, 2004) and IL-12 signaling (Kitamura *et al.*, 1999), resulting in DC maturation. We first examined the effects of treatments of DCs using lipopolysaccharides or CpG oligonucleotides to induce DC maturation *in vitro* before injection to mice. We did not observe significant enhancement of immune responses by these treatments (data not shown). We consider that immunization with αGalCer-pulsed DCs is sufficient for activation of DC itself *in vivo* through feedback effects by activated *i*NKT cells.

αGalCer administration has been carried out as free αGalCer administration or as αGalCer-pulsed DC administration. Free αGalCer administration induces the rapid increase of serum IFN-γ, IL-4 and IL-12 concentrations (Fujii *et al.*, 2002), but repeated αGalCer administration induces splenic T cells to secrete IL-4 and IL-10, and dramatically reduced levels of IFN-γ (Burdin *et al.*, 1999). In addition, αGalCer presentation by B cells suppresses DC–*i*NKT interaction when αGalCer is given as a free drug (Bezbradica *et al.*, 2005). Conversely, immunization with αGalCer-loaded DCs induces sustained IFN-γ production (Fujii *et al.*, 2002). Our experiments described here show that after the injection of DCs pulsed with αGalCer at priming phase, the serum IFN-γ level was significantly elevated. *i*NKT cells produce large amounts of IFN-γ and the production requires direct contact between *i*NKT cells and DCs through CD40–CD40 ligand interactions (Nishimura *et al.*, 2000). IFN-γ produced by *i*NKT cells has been shown to have a critical role in the antimetastatic effect of αGalCer in murine tumor models (Hayakawa *et al.*, 2001; Smyth *et al.*, 2002). The rapid IFN-γ production has been reported to stimulate the development of $CD8^+$ T-cell responses strongly, and also regulates $CD8^+$ T-cell contraction, and subsequently affects the formation of $CD8^+$ T-cell memory (Whitmire *et al.*, 2005). This finding is supported by our results that strong stimulation with DCs pulsed with αGalCer at priming phase but not at boosting phase leads to rapid IFN-γ production and induced stronger *in vivo* CTL activity. It is also conceivable that in addition to IFN-γ, direct DC activation by *i*NKT cells through CD40 signaling may affect the subsequent $CD8^+$ T-cell responses (Hermans *et al.*, 2003). Here, we showed that immunization with DCs pulsed with αGalCer together with an antigenic peptide at priming phase induced more $CD44^+$ memory-phenotype $CD8^+$ T cells, indicating that effective and strong priming of naïve T cells was elicited by αGalCer on DCs.

Another important finding here is that the consecutive use of αGalCer on DC immunization at both priming and boosting phases had deleterious effects on the effective memory T-cell response. *In vivo* CTL activity and the

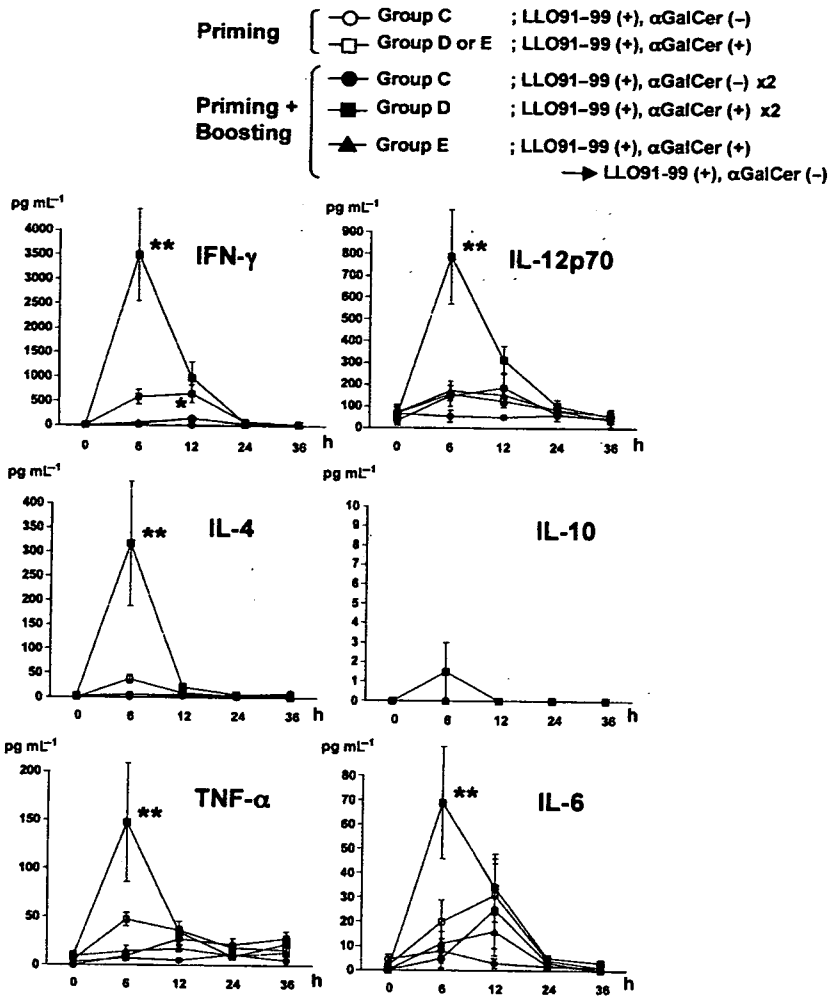

Fig. 6. Induction of cytokines in sera of LLO91-99 peptide and/or αGalCer-pulsed DC-immune mice. Sera were obtained from the immune mice at indicated time points after priming and boosting DC injections. Amounts of IFN-γ, IL-12p70, IL-4, IL-10, TNF-α and IL-6 in sera of the immune mice were evaluated with a cytometric bead array. Open symbols indicate cytokine levels after priming DC injection, and closed symbols indicate those after boosting DC injection. The means ± SE of five mice per group at each time point are shown. Single asterisk and double asterisks indicate significant differences of $P < 0.05$ and $P < 0.01$ compared with other groups at the same time point, respectively.

bacterial load in the spleens of *Listeria*-challenged mice, and also central memory $CD8^+$ T-cell pool formation were diminished by the consecutive use of αGalCer on DC immunization. Recent studies indicated that $CD8^+$ central memory T cells, but not $CD8^+$ effector memory T cells, play a pivotal role in protective immunity against the pathogen (Wherry *et al.*, 2003; Zaph *et al.*, 2004; Roberts *et al.*, 2005; Vaccari *et al.*, 2005). Therefore, diminished central memory $CD8^+$ T-cell formation in group D may reduce antilisterial immunity. Serum levels of Th1-type cytokines (IFN-γ and IL-12p70) were mostly elevated after the boost immunization in mice given DCs pulsed with LLO91-99 peptide and αGalCer at both phases (group D) (Fig. 6). In addition, serum levels of inflammatory cytokines (TNF-α and IL-6) and Th2-type cytokine (IL-4) were also elevated in the same group of mice, although the Th1-dominant immune response was maintained even at boosting phase (Fig. 6). Recently, Badovinac *et al.* (2005) reported that LLO91-99 peptide-pulsed DC vaccination generated $CD8^+$ T cells with the phenotype and function of memory cells within 4–6 days, leading to elevated numbers of effector and memory T cells and enhanced protective immunity compared with immunization with a sublethal dose of *L. monocytogenes*. In their system, they showed that inflammation events, such as infection with a sublethal dose of *L. monocytogenes* or CpG treatment, which lead to IFN-γ production, prevented the accelerated generation of memory $CD8^+$ T cells. Although early inflammatory signals, including IFN-γ production, are required to mediate activation of immature DCs and promote Ag presentation to naïve T cells, inflammatory

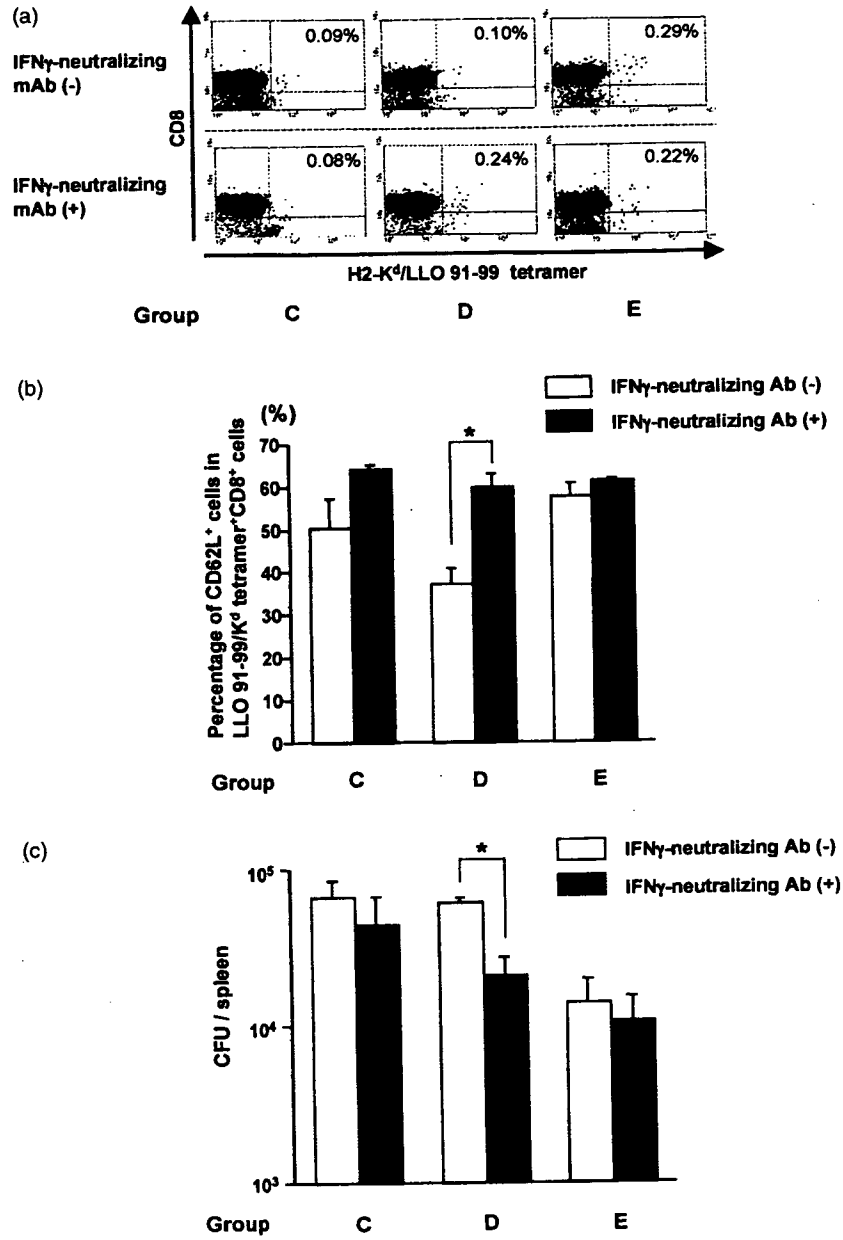

Fig. 7. Blockade of IFN-γ at boosting phase in mice given αGalCer-pulsed DC at both priming and boosting phases ameliorated specific CD8$^+$ T-cell induction, specific central memory T-cell formation, and induction of antilisterial immunity. Anti-IFN-γ neutralizing mAb was administered 2 days before boosting immunization. (a) Three days after boosting immunization, the spleen cells from the immune mice were prepared and analyzed for the appearance of H2-K^d/LLO91-99 tetramer$^+$ CD8$^+$ T cells with flow cytometry. The percentages of the tetramer$^+$ cells in CD8$^+$ T cells of each experimental group are shown. Representative data are shown. (b) The percentage of CD62L$^+$ cells in H2-K^d/LLO91-99 tetramer$^+$ CD8$^+$ T cells was examined 4 weeks after boost immunization. The asterisk indicates a significant difference ($P < 0.05$). (c) *In vivo* protection assay of antimouse IFN-γ mAb-treated mice against lethal *L. monocytogenes* challenge infection. The means ± SE of three mice per group are shown. Asterisks indicate significant differences ($P < 0.05$).

signals in the late phase of the immune response may act to enhance the proliferation and also the contraction of the T-cell response through apoptosis, leading to a delay in the generation of stable T-cell memory (Badovinac *et al.*, 2005; Woodland & Blackman, 2005). Blocking IFN-γ with the neutralizing mAb at boosting phase in mice given DCs pulsed with αGalCer at priming and boosting phases improved CTL responses, the formation of specific central memory T-cell pool and the reduction of the bacterial load in the spleens of *Listeria*-challenged mice. This suggests that

excessive amounts of IFN-γ at boosting phase deteriorate CTL responses and generation of the central memory T-cell pool. Too much inflammation and the subsequent induction of specific CTL would eliminate Ag-loaded APCs, including DCs at boosting phase, and the elimination would limit the sustenance of effective T-cell responses (Hermans *et al.*, 2000; Ronchese & Hermans, 2001). The prevention of the elimination of APCs at the boosting phase might be important for the induction of an effective stable T-cell memory. Too much inflammation would also induce activation-induced cell death of CTL (Woodland & Blackman, 2005). The results described here suggest that the removal of inflammatory signals at boosting phase may be important for an effective vaccine strategy. Although administration of αGalCer was often repeated for the enhancement of tumor immunotherapy (Ishikawa *et al.*, 2005), the deleterious effect of αGalCer at boosting phase should be taken into account. It would be preferable to add an additional combination group-LLO91-99 peptide (+)/αGalCer (−) at priming phase and LLO91-99 peptide (+)/αGalCer (+) at boosting phase to confirm the deleterious effect of αGalCer at boosting phase on peptide-pulsed DC immunization, although we were not able to add the group in this study.

The result described here that the immunization of Ag peptide-pulsed DCs with αGalCer at priming phase, but not at boosting phase, is feasible for an effective specific $CD8^+$ T-cell memory pool and the induction of protective immunity will shed light on effective vaccination strategies for specific $CD8^+$ T-cell induction. This effective immunization method using αGalCer-treated DCs would be also applicable to tumor immunotherapy.

Acknowledgements

We thank Dr M. Mitsuyama (Kyoto University, Japan) for generously providing the *L. monocytogenes* strain EGD. This work was supported by Grants-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science [11670260 (T.N.), 13670268 (Y.K.), 13670595 (T.S.)], a Grant-in-Aid for Center of Excellence (COE) research program from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan, and also a Grant-in-Aid from the United States–Japan Cooperative Medical Science Program.

References

Ahuja SS, Reddick RL, Sato N, Montalbo E, Kostecki V, Zhao W, Dolan MJ, Melby PC & Ahuja SK (1999) Dendritic cell (DC)-based anti-infective strategies: DCs engineered to secrete IL-12 are a potent vaccine in a murine model of an intracellular infection. *J Immunol* **163**: 3890–3897.

Aichele P, Brduscha-Riem K, Oehen S, Odermatt B, Zinkernagel RM, Hengartner H & Pircher H (1997) Peptide antigen treatment of naive and virus-immune mice: antigen-specific tolerance versus immunopathology. *Immunity* **6**: 519–529.

Badovinac VK, Messingham AN, Jabbari A, Haring JS & Harty JT (2005) Accelerated $CD8^+$ T-cell memory and prime-boost response after dendritic-cell vaccination. *Nat Med* **11**: 748–756.

Banchereau J & Steinman RM (1998) Dendritic cells and the control of immunity. *Nature* **392**: 245–252.

Bezbradica JS, Stanic AK, Matsuki N, Bour-Jordan HJ, Bluestone A, Thomas JW, Untmaz D, Kaer LV & Joyce S (2005) Distinct roles of dendritic cells and B cells in Va14Ja18 natural T cell activation *in vivo*. *J Immunol* **174**: 4696–4705.

Burdin N, Brossay L & Kronenberg M (1999) Immunization with α-galactosylceramide polarizes CD1-reactive NK T cells towards Th2 cytokine synthesis. *Eur J Immunol* **29**: 2014–2025.

Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT & Falo LD Jr (1996) Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. *J Exp Med* **183**: 283–287.

Crowe NY, Uldrich AP, Kyparissoudis K, Hammond KJL, Hayakawa Y, Sidobre S, Keating R, Kronenberg M, Smyth MJ & Godfrey DI (2003) Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells. *J Immunol* **171**: 4020–4027.

Emoto M, Emoto Y, Buchwalow IB & Kaufmann SHE (1999) Induction of IFN-γ-producing $CD4^+$ natural killer T cells by *Mycobacterium bovis* bacillus Calmette-Guérin. *Eur J Immunol* **29**: 650–659.

Fujii S, Shimizu K, Kronenberg M & Steinman RM (2002) Prolonged IFN-γ-producing NKT response induced with α-galactosylceramide-loaded DCs. *Nat Immunol* **3**: 867–874.

Fujii S, Liu K, Smith C, Bonito AJ & Steinman RM (2004) The linkage of innate to adaptive immunity via maturing dendritic cells *in vivo* requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J Exp Med* **199**: 1607–1618.

Gelin BG & Broome CV (1989) Listeriosis. *JAMA* **261**: 1313–1320.

Gonzalez-Aseguinolaza G, de Oliveira C, Tomaska M *et al.* (2000) α-Galactosylceramide-activated Vα14 natural killer T cells mediate protection against murine malaria. *Proc Natl Acad Sci USA* **97**: 8461–8466.

Gumperz JE & Brenner MB (2001) CD1-specific T cells in microbial immunity. *Curr Opin Immunol* **13**: 471–478.

Harada M, Seino K, Wakao H, Sakata S, Ishizuka Y, Ito T, Kojo S, Nakayama T & Taniguchi M (2004) Down-regulation of the invariant Vα14 antigen receptor in NKT cells upon activation. *Int Immunol* **16**: 241–247.

Harty JT & Bevan MJ (1992) $CD8^+$ T cells specific for a single nonamer epitope of *Listeria monocytogenes* are protective *in vivo*. *J Exp Med* **175**: 1531–1538.

Hayakawa Y, Takeda K, Yagita H, Kakuta S, Iwakura Y, Kaer LV, Saiki I & Okumura K (2001) Critical contribution of IFN-γ and NK cells, but not perforin-mediated cytotoxicity to anti-metastatic effect of α-galactosylceramide. *Eur J Immunol* **31**: 1720–1727.

Hermans IF, Ritchie DS, Yang J, Roberts JM & Ronchese F (2000) $CD8^+$ T cell-dependent elimination of dendritic cells *in vivo* limits the induction of antitumor immunity. *J Immunol* **164**: 3095–3101.

Hermans IF, Silk JD, Gileadi U, Salio M, Mathew B, Ritter G, Schmidt R, Harris AL, Old L & Cerundolo V (2003) NKT cells enhance $CD4^+$ and $CD8^+$ T cell responses to soluble antigen *in vivo* through direct interaction with dendritic cells. *J Immunol* **171**: 5140–5147.

Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Muramatsu S & Steinman RM (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* **176**: 1693–1702.

Ishikawa H, Hisaeda H, Taniguchi M *et al.* (2000) $CD4^+$ Vα14 NKT cells play a crucial role in an early stage of protective immunity against infection with *Leishmania major*. *Int Immunol* **12**: 1267–1274.

Ishikawa A, Motohashi S, Ishikawa E, Fuchida H, Higashino K, Otsuji M, Iizasa T, Nakayhama T, Taniguchi M & Fujisawa T (2005) A phase I study of α-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer. *Clin Cancer Res* **11**: 1910–1917.

Kakimi K, Guidotti LG, Koezuka Y & Chisari FV (2000) Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med* **192**: 921–930.

Kikuchi T, Worgall S, Singh R, Moore MA & Crystal RG (2000) Dendritic cells genetically modified to express CD40 ligand and pulsed with antigen can initiate antigen-specific humoral immunity independent of $CD4^+$ T cells. *Nat Med* **6**: 1154–1159.

Kitamura H, Iwakabe K, Yahata T *et al.* (1999) The natural killer (NKT) cell ligand α-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J Exp Med* **189**: 1121–1127.

Kronenberg M (2005) Toward an understanding of NKT cell biology: progress and paradoxes. *Annu Rev Immunol* **23**: 877–900.

Kronenberg M & Gapin L (2002) The unconventional lifestyle of NKT cells. *Nat Rev Immunol* **2**: 557–568.

Lefrançois L & Masopust D (2002) T cell immunity in lymphoid and non-lymphoid tissues. *Curr Opin Immunol* **14**: 503–508.

Manickan E, Kanangat S, Rouse RJ, Yu Z & Rouse BT (1997) Enhancement of immune response to naked DNA vaccine by immunization with transfected dendritic cells. *J Leukoc Biol* **61**: 125–132.

Mayordomo JI, Zorina T, Stokus WJ *et al.* (1995) Bone marrow-derived dendritic cells pulsed with synthetic tumor peptides elicit protective and therapeutic antitumor immunity. *Nat Med* **1**: 1297–1302.

Nakamura Y, Suda T, Nagata T, Aoshi T, Uchijima M, Yoshida A, Chida K, Koide Y & Nakamura H (2003) Induction of protective immunity to *Listeria monocytogenes* with dendritic cells retrovirally transduced with a cytotoxic T lymphocyte epitope minigene. *Infect Immun* **71**: 1748–1754.

Nishimura T, Kitamura H, Iwakabe K *et al.* (2000) The interface between innate and acquired immunity: glycolipid antigen presentation by CD1d-expressing dendritic cells to NKT cells induces the differentiation of antigen-specific cytotoxic T lymphocytes. *Int Immunol* **12**: 987–994.

Pamer EG (2004) Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol* **4**: 812–823.

Ranieri E, Herr W, Gambotto A, Olson W, Rowe D, Robbins PD, Salvucci Kierstead L, Watkins SC, Gesualdo L & Storkus WJ (1999) Dendritic cells transduced with an adenovirus vector encoding Epstein-Barr virus latent membrane protein 2B: a new modality for vaccination. *J Virol* **73**: 10416–10425.

Ranson T, Bregenholt S, Lehuen A, Gaillot O, Leite-de-Moraes MC, Herbelin A, Berche P & Di Santo JP (2005) Invariant $V\alpha14^+$ NKT cells participate in the early response to enteric *Listeria monocytogenes* infection. *J Immunol* **175**: 1137–1144.

Roberts AD, Ely KH & Woodland DL (2005) Differential contributions of central and effector memory T cells to recall responses. *J Exp Med* **202**: 123–133.

Ronchese F & Hermans IF (2001) Killing of dendritic cells: a life cut short or a purposeful death? *J Exp Med* **194**: F23–F26.

Seder RA & Hill AV (2000) Vaccines against intracellular infections requiring cellular immunity. *Nature* **406**: 793–798.

Shaw JH, Grund VR, Durling L & Caldwell HD (2001) Expression of genes encoding Th1 cell-activating cytokines and lymphoid homing chemokines by *Chlamydia*-pulsed dendritic cells correlates with protective immunizing efficacy. *Infect Immun* **69**: 4667–4672.

Silk JD, Hermans IF, Gileadi U *et al.* (2004) Utilizing the adjuvant properties of CD1d-dependent NK T cells in T cell-mediated immunotherapy. *J Clin Invest* **114**: 1800–1811.

Smyth MJ, Crowe NY, Pellicci DG, Kyparissoudis K, Kelly JM, Takeda K, Yagita H & Godfrey DI (2002) Sequential production of interferon-γ by $NK1.1^+$ T cells and natural killer cells is essential for the antimetastatic effect of α-galactosylceramide. *Blood* **99**: 1259–1266.

Uchijima M, Yoshida A, Nagata T & Koide Y (1998) Optimization of codon usage of plasmid DNA vaccine is required for the effective MHC class I-restricted T cell responses against an intracellular bacterium. *J Immunol* **161**: 5594–5599.

Vaccari M, Trindade CJ, Venzon D & Zanetti M (2005) Vaccine-induced $CD8^+$ central memory T cells in protection from simian AIDS. *J Immunol* **175**: 3502–3507.

Wherry EJ, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, von Andrian UH & Ahmed R (2003) Lineage

relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* **4**: 225–234.

Whitmire JK, Tan JT & Whitton JL (2005) Interferon-γ acts directly on $CD8^+$ T cells to increase their abundance during virus infection. *J Exp Med* **201**: 1053–1059.

Wilson MT, Johansson C, Olivares-Villagómez D, Singh AK, Stanic AK, Wang C-R, Joyce S, Wick MJ & Kaer LV (2003) The response of natural killer T cells to glycolipid antigens is characterized by surface receptor down-modulation and expansion. *Proc Natl Acad Sci USA* **100**: 10913–10918.

Woodland DL & Blackman MA (2005) Vaccine development: baring the 'dirty little secret'. *Nat Med* **11**: 715–716.

Zaph C, Uzonna J, Beverley SM & Scott P (2004) Central memory T cells mediate long-term immunity to *Leishmania major* in the absence of persistent parasites. *Nat Med* **10**: 1104–1110.

リファンピシンを含む結核治療におけるシクロスポリン投与量の検討

[1,2]松井 芳憲 [1]赤川志のぶ [1]川島 正裕 [1]鈴木 純子
[1]益田 公彦 [1]田村 厚久 [1]永井 英明 [1]長山 直弘
[1]川辺 芳子 [1]町田 和子 [1]倉島 篤行 [1]四元 秀毅

要旨：〔目的〕すでにプレドニゾロン（PSL）やシクロスポリン（CyA）が投与されている結核患者にRFPを投与する場合，これら先行薬剤の増量を要する。PSLは2倍量程度が推奨されているが，CyAについては未だ定説がない。そこでRFP投与時のCyAの必要投与量を知るために，CyAがすでに投与されていた結核患者における投与量について検討した。〔方法と結果〕2001年から2003年の間に当院でRFPを含む肺結核の化学療法を行った患者のうち，治療前からCyAを使用していた4例の患者において結核治療前と同等のトラフ値が得られたCyAの投与量を検討した。全例PSLが投与されており，最初から2倍に増量されていた。CyAはトラフ値を測定して投与量を調節されており，2.5～3.5（平均3）倍量で結核治療開始前のトラフ値に到達し，臨床所見も安定した。CyA投与量の決定までに，トラフ値は5～12（平均8.3）回測定され，6～27（平均12）週を要していた。〔結論〕CyAの投与量決定には長期間，頻回のトラフ値測定を要したが，3倍量前後で目標値に達していた。したがって結核治療の最初からCyAは3倍に増量すると効率がよいと思われた。

キーワーズ：リファンピシン，シクロスポリン，プレドニゾロン，薬物相互作用，結核

はじめに

リファンピシン(RFP)は，主にチトクローム P450ⅢA（CYP3A）系酵素を誘導し，プレドニゾロン（PSL）やシクロスポリン（CyA）と併用した場合，これらの代謝を促進し半減期を短縮させる。したがってPSLやCyA投与患者に抗結核療法を行う場合は薬剤の増量を必要とする。

PSLは2倍量程度の増量が推奨されているが[1)2)]，CyAについては約3～5倍に増量[3)]や約2.5～3倍に増量[4)]したとの報告があるが，いずれも海外での報告であり，本邦の報告は未だない。

そこで今回われわれは，CyA使用中に発症した肺結核患者において，RFP併用後のCyAの投与量変更がどのようになされていたのかを検討したので報告する。

対象と方法

2001年から2003年の間に当院でRFPを含む肺結核の化学療法を行った患者のうち，すでにCyAを使用していた4例について後ろ向きに検討した。内訳は，男性2例，女性2例，体重46～63 kg，年齢51～78歳，間質性肺炎2例，全身性エリテマトーデス（SLE）1例，強皮症1例である。治療レジメは，イソニアジド（INH）＋RFP＋エタンブトール（EB）3剤（HRE）が3例，HRE＋ピラジナミド（PZA）4剤（HREZ）が1例である。全例RFPは450 mg/日で投与されていた。

これら4例についてCyAの投与量変更の方法と臨床経過について検討した。

結　果

全例，PSLが併用されており，1日1回投与であった。

[1]独立行政法人国立病院機構東京病院呼吸器内科，[2]東京慈恵会医科大学呼吸器内科

連絡先：松井芳憲，独立行政法人国立病院機構東京病院呼吸器内科，〒204-8585 東京都清瀬市竹丘3-1-1
(E-mail: yoshi-matsui@nifty.com)
(Received 11 Jan. 2007/Accepted 15 Mar. 2007)

CyAは1日2分割で投与されていた。PSLは，全例最初から2倍量投与に変更されていた。CyAについてはRFP開始前のCyAトラフ値を目標として，トラフ値を測定しながら投与量の変更が行われていた。

以下，症例ごとに経過を示す。

症例1（Fig. 1）：69歳女性。平成8年より強皮症・間質性肺炎に対してPSL開始され，平成14年よりCyA併用となり，PSL 7.5 mg/日，CyA 100 mg/日使用しCyAトラフ値100 ng/ml前後でコントロール良好であった。平成15年6月肺結核（bⅡ1）発症し，抗結核薬としてHREを開始。PSLを15 mg/日に増量，CyAを200 mg/日に増量したが，CyAトラフ値は50 ng/ml前後と低値を示した。CyAトラフ値測定を繰り返し，最終的に7週後にCyA 300 mg/日使用しCyAトラフ値100 ng/ml前後でコントロール良好となった。その時点までにトラフ値測定は8回行われていた。

症例2（Fig. 2）：78歳男性。平成16年3月間質性肺炎急性増悪に対して治療開始され，PSL 20 mg/日，CyA 150 mg/日使用しCyAトラフ値100 ng/ml前後でコントロール良好であった。同年6月肺結核（bⅢ2）発症し，抗結核薬としてHREを開始。PSLを40 mg/日に増量，CyAを250 mg/日に増量したが，CyAトラフ値40 ng/ml前後と低値のため，CyAをさらに増量した。経過中，薬疹出現にてHREを一時休薬し，INH+EBとレボフロキサシン（LVFX）に変更し，RFPは減感作成功後に常用量に戻した。最終的に12週後にCyA 450 mg/日使用しCyAトラフ値100 ng/ml前後となりコントロール良好となった。その時点までにトラフ値測定は8回行われて

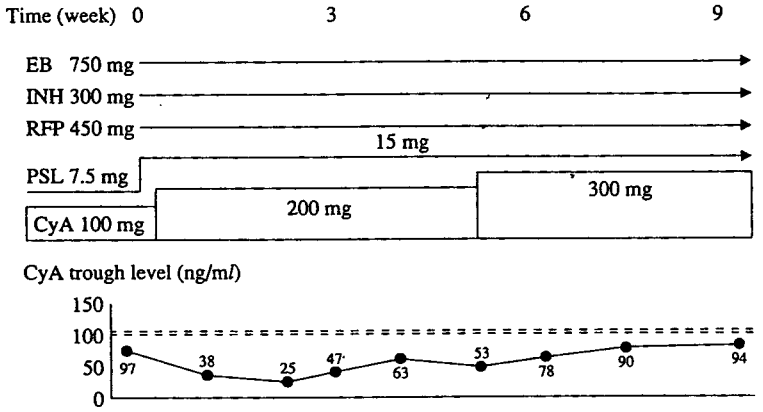

Fig. 1 Daily dose of CyA during anti-tuberculosis treatment containing RFP to maintain optimum CyA trough level (case 1: a 69-year-old woman with scleroderma and interstitial pneumonia)
EB: ethambutol, INH: isoniazid, RFP: rifampicin, PSL: prednisolone, CyA: cyclosporine

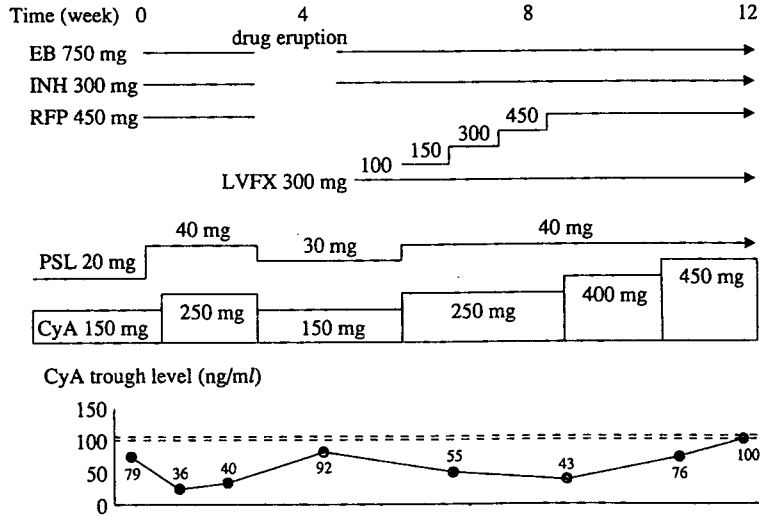

Fig. 2 Daily dose of CyA during anti-tuberculosis treatment containing RFP to maintain optimum CyA trough level (case 2: a 78-year-old man with idiopathic pulmonary fibrosis)
LVFX: levofloxacin

いた。

症例3（Fig. 3）：51歳男性。平成15年5月間質性肺炎急性増悪に対して治療開始され，PSL 15 mg/日，CyA 200 mg/日使用し，CyAトラフ値100 ng/ml前後でコントロール良好であった。同年7月肺結核（bⅢ2）発症し，抗結核薬としてHREを開始。本例はPSL減量中であったため，一時的にPSLを30 mg/日に増量してから，再び減量を行った。CyAトラフ値は安定せず，CyAの増量を繰り返した。最終的に27週後CyA700 mg/日使用しCyAトラフ値100 ng/ml前後でコントロール良好となった。その時点までにトラフ値測定は12回行われていた。

症例4（Fig. 4）：71歳女性。平成15年よりSLEにてPSL開始され，平成16年よりCyA併用となり，PSL 30 mg/日，CyA 100 mg/日にてCyAトラフ値40 ng/ml前後でコントロール良好であった。同年7月肺結核（bⅢ3）発症し，抗結核薬としてHREZを開始。本例もPSL減量中にて一時的にPSL 60 mg/日に増量してから減量を行った。CyAは150 mg/日に増量したがCyAトラフ値30 ng/ml前後と低値のため，さらに増量した。最終的に6週後にCyA 250 mg/日使用しCyAトラフ値40 ng/ml前後でコントロール良好となった。その時点までにトラフ値測定は5回行われていた。

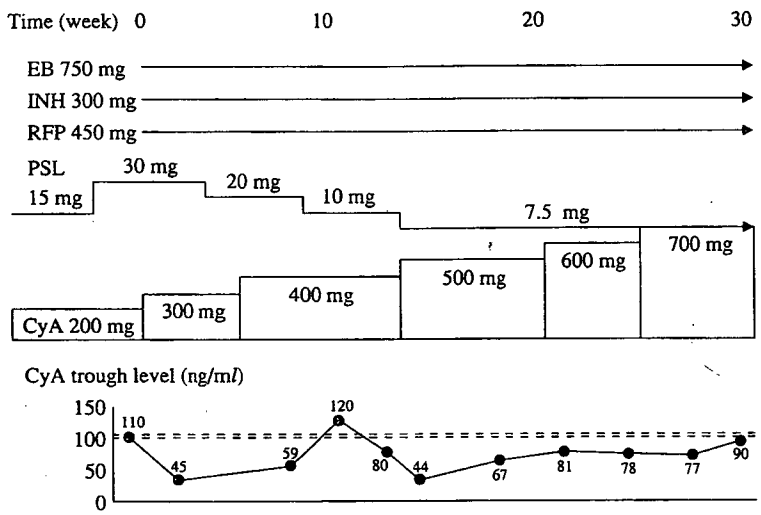

Fig. 3 Daily dose of CyA during anti-tuberculosis treatment containing RFP to maintain optimum CyA trough level (case 3: a 51-year-old man with idiopathic pulmonary fibrosis)

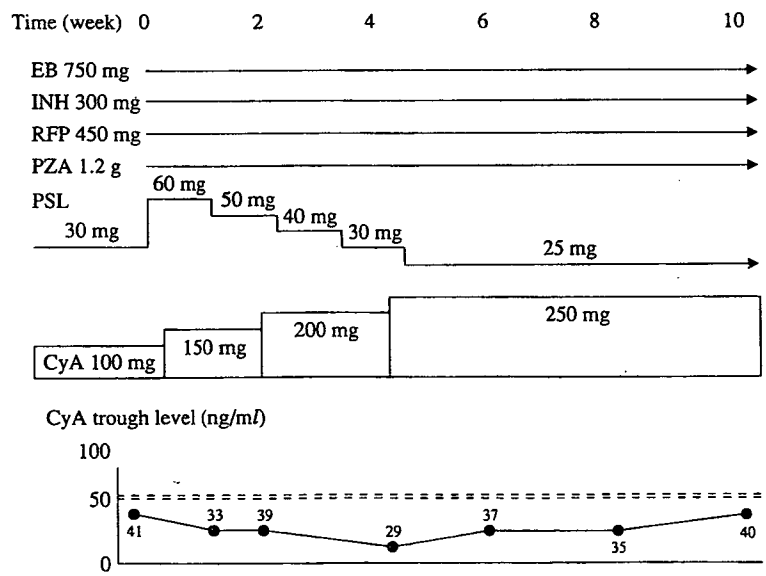

Fig. 4 Daily dose of CyA during anti-tuberculosis treatment containing RFP to maintain optimum CyA trough level (case 4: a 71-year-old woman with systemic lupus erythematodes)

Table Clinical data of 4 patients about cyclosporine

Case	Sex	Age (yr)	RFP dose (mg/day)	RFP (−) CyA dose (mg/day)	RFP (+) final CyA dose (mg/day)	To reach final CyA trough levels	
						Frequency of measurement (times)	Periods (week)
1	F	69	450	100	300	8	7
2	M	78	450	150	450	8	12
3	M	51	450	200	700	12	27
4	F	71	450	100	250	5	6
					Average	8.3	13

CyA: cyclosporine, RFP: rifampicin

4症例とも肺結核の経過は順調で，全例化療開始1カ月後に菌陰性化した。また，いずれの症例においても原疾患の悪化，副作用は認めなかった。

以上をまとめると（Table），結核化療開始時にPSLは全例で2倍に増量されていた。一方CyAは化療開始時に1.5～2倍に増量された後，投与量の変更を頻回に繰り返されていた。最終的にCyAは2.5～3.5（平均3）倍量でトラフ値が目標値（化療開始前の値）に達し，投与量が定まった。CyAトラフ値測定は5～12（平均8.3）回施行され，安定までに6～27（平均13）週を要していた。

考　　案

近年ステロイド，免疫抑制剤使用の増加に伴いimmunocompromised hostも多くなっている。今回の4例も基礎疾患に間質性肺炎や膠原病があり，ステロイドと免疫抑制剤CyA投与中に日和見感染として肺結核が発症し，RFPを含む抗結核療法が開始されている。このような場合問題となるのが，ステロイドおよびCyAとRFPとの薬物相互作用である。RFPは，肝臓におけるチトクロームP450系の様々なアイソザイムをふくむ多様な代謝経路を誘導する物質として知られている[1)~3)]。RFP投与により代謝酵素の活性が誘導され，薬剤によっては代謝を受けることにより血中濃度が低下し，その薬剤として期待されていた治療域に達しないことが生じる[4)]。また，投与開始から肝酵素が誘導されるまでに数日から2週間ほどかかり，投与を中止しても影響が消失するまでにも同様の日数を要するとされている。

ステロイド類はRFPの併用により半減期が短縮するといわれている。ステロイドの種類により短縮率は異なるが，PSLにおいては短縮率44%で，必要量は2倍になるという報告がある[5)6)]。今回のわれわれの結果においても，PSLはこれまでの推奨どおり全例2倍量に増量されていた。

一方CyAに関しては，1990年にAl-Sulaimanらにより腎移植患者6例において約3～5倍に増量したという報告と[7)]，1998年にKimらにより腎移植患者4例において約2.5～3倍に増量したという報告がなされている[8)]。いずれも海外の腎移植患者におけるRFP 600 mg/日併用時の報告であり，多くがRFP 450 mg/日を使用している本邦での報告は未だない。

免疫抑制剤CyAは，主としてT細胞（ヘルパーT細胞）によるインターロイキン-2（IL-2）などのサイトカイン産生を阻害することにより強力な免疫抑制作用を示すとされているが，免疫抑制効果を期待できる血中濃度と中毒域との差が小さい。そのためCyAを効果的に用いるには，トラフ値で代表されるように全血で血中濃度をモニタリングして投与量を決定しなければならない[9)]。RFPとの併用開始後，RFPの影響が最大となるまでに数日から2週間程度を要し，RFP中止4～7日後までCyA濃度の低下が認められ，CyAの濃度回復に平均10日を要すると報告されている[10)]。また，RFPとの併用開始後CyAの血中濃度が低下し原疾患の悪化を認めたとの報告もある[11)]。したがって，RFPとの併用時には慎重にCyAの投与量を決める必要がある。

今回のわれわれの検討では，RFP 450 mg/日併用時にはCyAは最終的に投与量を2.5～3.5倍（平均3倍）に増量することにより，CyAトラフ値がRFP投与前の値に到達し，臨床的にも安定した状態を保つことができた。また，RFP開始しCyA増量後6～12日（平均7日）後には，CyAトラフ値がRFP投与前の値に到達した。しかしながら，CyA投与量決定までに6～27週（平均13週），CyAトラフ値測定も5～12回（平均8.3回）を要しており，きわめて非効率的であった。これらの結果より本邦においては，RFP併用開始時にはCyAを数日～2週間以内に3倍量に増量し，約7日後のCyAトラフ値を参考にして微調整すれば効率よく適切なCyA投与量を決めることが可能と思われた。

文　　献

1) Bachmann KA, Jauregui L: Use of single sample clearance estimates of cytochrome P450 substrates to characterize human hepatic CYP status *in vivo*. Xenobiotica. 1993 ; 23 : 307–315.

2) Caraco Y, Sheller J, Wood AJ: Pharmacogenetic Determination of Codeine Induction by Rifampin: The Impact on Codeine's Respiratory, Psychomotor and Miotic Effects. 1997 ; 281 : 330–336.
3) Dilger K, Greiner B, Fromm MF, et al.: Consequences of rifampicin treatment on propafenone disposition in extensive and poor metabolizers of CYP2D6. Pharmacogenetics. 1999 ; 9 : 551–559.
4) Strayhorn VA, Baciewicz AM, Self TH: Update on Rifampin Drug Interactions, Ⅲ. Arch Intern Med. 1997 ; 157 : 2453–2458.
5) 河合眞一, 市川陽一, 本間光夫：リファンピシン併用によりステロイド治療抵抗性を示した膠原病症例とその薬物速度論的分析. リウマチ. 1984 ; 24 : 32–37.
6) 河合眞一：リファンピシン服用者における各種糖質コルチコイド代謝動態の比較. 日内分泌会誌. 1985 ; 61 : 145–161.
7) Al-Sulaiman MH, Dhar JM, Al-Khader AA: Successful use of rifampicin in the treatment of tuberculosis in renal transplant patients immunosuppressed with cyclosporine. Transplantation. 1990 ; 50 : 597–598.
8) Kim YH, Yoon YR, Kim YW, et al.: Effects of rifampin on cyclosporine disposition in kidney recipients with tuberculosis. Transplantation Proceedings. 1998 ; 30 : 3570–3572.
9) Hebert MF, Roberts JP: Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. J Clin Pharmacol. 1992 ; 52 : 453–457.
10) Zelunka EJ: Intravenous Cyclosporine-Rifampin Interaction in a Pediatric Bone Marrow Transplant Recipient. Pharmacotherapy. 2002 ; 22 : 387–390.
11) Offermann G, Keller F, Molzahn M: Low cyclosporine blood levels and acute graft rejection in a renal transplant patient recipient during rifampin treatment. Am J Nephrol.

Original Article

EXAMINATION OF ADMINISTRATIVE DOSAGE OF CYCLOSPORINE DURING THE ANTI-TUBERCULOSIS CHEMOTHERAPY INCLUDING RIFAMPICIN

[1,2]Yoshinori MATSUI, [1]Shinobu AKAGAWA, [1]Masahiro KAWASHIMA, [1]Junko SUZUKI, [1]Kimihiko MASUDA, [1]Atsuhisa TAMURA, [1]Hideaki NAGAI, [1]Naohiro NAGAYAMA [1]Yoshiko KAWABE, [1]Kazuko MACHIDA, [1]Atsuyuki KURASHIMA, and [1]Hideki YOTSUMOTO

Abstract [Aims] In the treatment of tuberculosis with rifampicin in patients treated with prednisolone and cyclosporine, we have to increase the dosage of these drugs. Although prednisolone dosage is recommended to be doubled, there is no established consensus about cyclosporine dosage. Our aim is to review the current situation at our institution regarding the dosage of cyclosporine administered to tuberculous patients after the addition of rifampicin to the treatment regimen.

[Methods and Results] We reviewed patients' clinical status and how dosages of cyclosporine were altered during a course of tuberculosis treatment including rifampicin in 4 patients (2 interstitial pneumonitis, 2 collagen vascular disease) who were being treated with cyclosporine between 2001 and 2003. Prednisolone had been also administrated in all patients and the dosage was doubled from the beginning of the treatment. The appropriate dosage of cyclosporine was found to be 2.5–3.5 (average 3) times that of initial dosage, and it required 5–12 weeks (average 8.3) measurements of trough levels and 6–27 (average 12) weeks until appropriate trough levels were obtained.

[Conclusions] The appropriate dosage of cyclosporine was found to be approximately 3 times that of the initial dosage in all patients, but it required a long term and frequent measurements of trough levels before reaching this goal. It seems that trebling the dosage of cyclosporine from the start of anti-tuberculosis chemotherapy will be an efficient way to achieve good clinical outcome.

Key words: Rifampicin, Cyclosporine, Prednisolone, Drug interactions, Tuberculosis

[1]Department of Pulmonary Medicine, National Hospital Organization Tokyo National Hospital, [2]Department of Pulmonary Medicine, Jikei University School of Medicine

Correspondence to: Yoshinori Matsui, Department of Pulmonary Medicine, National Hospital Organization Tokyo National Hospital, 3–1–1, Takeoka, Kiyose-shi, Tokyo 204–8585 Japan. (E-mail: yoshi-matsui@nifty.com)

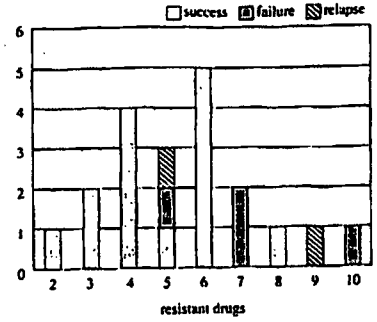

Fig. Resistant drugs and results of incomplete resections

Table 2 Background of incomplete resections

		Preoperative bacilli within 3 months	Resistant drugs	Residual lesion	Postoperative chemotherapy months
Success	1	none	3	nodular/infiltration	24
	2	none	4	nodular/infiltration	19
	3	none	6	nodular/infiltration	13
	4	culture positive	4	nodular/infiltration	52
	5	culture positive	8	nodular/infiltration	22
	6	I	4	nodular/infiltration	6
	7	II	6	cavity	30
	8	III	5	nodular/infiltration	12
	9	V	6	nodular/infiltration	unknown
	10	V	6	nodular/infiltration	19
	11	VI	4	nodular/infiltration	6
	12	VI	6	nodular/infiltration	8
	13	VII	3	nodular/infiltration	24
	14	VII	2	nodular/infiltration	21
Failure	15	none	10	nodular/infiltration	9
	16	culture positive	5	nodular/infiltration	24
	17	VII	7	nodular/infiltration	25
	18	IX	7	nodular/infiltration	42
Relapse	19	none	5	nodular/infiltration	29
	20	VI	9	nodular/infiltration	29

1例，1個性病変1例（肺切除術予定であったが，理由は明らかでないが胸郭成形術なった症例）であった。術前3カ月の排菌状態は，全例が排菌陽性で，培養のみ陽性例が3例，塗抹培養とも陽性例が2例であった。術後の抗結核薬の投与期間は術後早期に死亡した1例を除くと24～120カ月であった。

（3）術後合併症

術後合併症は，肺切除31例中7例に，胸郭成形術5例中1例に発生した。そのうち，死亡に至った症例は3例であった。肺切除術後の死亡例は2例で，1例は術後膿胸を発生し非手術側肺に感染が波及したことによる結核死で，もう1例は術後の脳梗塞による死亡であった。胸郭成形術後の死亡例は，両側巨大空洞例の術後に肺結核が増悪した症例であった。死亡に至らなかった術後合併症はいずれも肺切除例に発生したものであり2例が術後出血，2例が膿胸，1例が術後は肺癌，1例は術後神経症状の出現であった。

考　察

肺結核の手術件数は，抗結核剤の開発に伴い著しく減少した。しかし，多剤耐性肺結核の場合，内科的治療で治癒しうる症例もあるものの，内科的治療のみでは治癒しきれない症例も存在する。多剤耐性肺結核で治療に失敗すると単に生命予後が不良であるばかりでなく，患者も長期入院を余儀なくされ著しくQOLも低下する。そして，患者個人のみでなく社会的にも経済的にもその損失は大きなものである。多剤耐性肺結核の治療を考える場合，内科的治療のみでなく常に外科的治療の可能性も考慮に入れておくことは重要であると思われる。今回，病巣を残さず切除し得たと思われる症例では，薬剤耐性数や術前の排菌状態に関係なく排菌を陰性化させることができた。このことより，手術可能な肺機能で病巣が限局化しており手術により完全に切除可能な多剤耐性肺結核は肺切除術が妥当であり，従来の報告のとおりであった[1][2]。また，術後の抗結核薬の投与も1年以上継続させればよいものと思われる。

肺切除症例のうち，病変を残した症例で術後再排菌を認めなかった症例は70%であった。残した病巣の性状は1例の空洞性病変を除き19例が粒状・浸潤影であった。また，治療失敗例，再発例の全例が遺残病変は粒状・浸潤影であった。中島は手術対象となる病変は硬化性空洞または径2cm以上の結核結節であるとしている[3]。井内らは再排菌，持続排菌の症例はそのほとんどが，遺残空洞性病変を有する症例であったと述べている[4]。しかし，今回の検討では，粒状・浸潤影でも病変を遺残させるとその30%で治療失敗や再発を認めている。病変を残した症例の薬剤耐性数は4種類以下では再排菌を認めていないが，5種類以上では治療失敗や再発を認められるようになり，7剤以上に耐性となると再排菌を認めない症例は20%に減少した。このことから，病変を残存させる可能性のある症例では耐性薬剤数が増加する前に手術に踏み切ることが必要と思われる。手術時期は，全身状態や薬剤治療の状況にもよると思われるが，十分な内科的治療を行ったうえで，4～6カ月たっても排菌が持続する場合が一般的と思われる[3][4]。今回の検討より，病変が完全に切除可能な場合は，手術により再排菌をしなくなる可能性が高いため，排菌が陰性化しても，肺機能などより十分手術に耐えうる症例は，相対的に肺切除術の適応となりうるものと思われる[3]。術後の抗結核薬の投与期間は，1年半から2年は必要と思われる[3]。しかし，今回の検討では術後2年以上抗結核薬投与を行っても再発した症例もあることより術後慎重な経過観察が必要と思われる。

胸郭成形術は肺切除術ができない症例に検討されるが，十分に症例を選択すればある程度はその治療的意義はあるものと思われる。しかし，肺切除術に比較すると治療効果は低いため術後長期間の抗結核薬の投与を必要とするものと考えられる。

まとめ

多剤耐性肺結核の手術では，病巣を完全に切除可能であれば排菌を停止させることができる可能性が高い。一方，病変を残さざるを得ない症例では，有効薬剤数が多いほど排菌を停止させうる可能性が高いので，内科的治療に抵抗性の場合は早期より外科的治療を検討することが重要である。

文　献

1）矢野　昇，荒井他喜明，福垣敬三，他：肺結核外科療法不成功例の検討．結核．1997；71：35-38.
2）小松彦太郎：結核症外科治療の適応とその有効性．肺結核「結核 Up to Date」，毛利昌史，四元秀毅，倉島篤行，南江堂，東京，1999，101-104.
3）中島由槻：多剤耐性結核の治療．結核．2002；77：805-813.
4）井内敬二，加田　元，中川静裕，他：多剤耐性肺結核の外科療法適応．THE LUNG perspectives．1999；7：402-405.
5）McLaughlin JS, Hankins JR：Current aspects of surgery for pulmonary tuberculosis. Ann Thorac Surg. 1974；17：513-525.

6. 多剤耐性結核患者に対する活性化T細胞輸注療法の試み

国立国際医療センター研究所呼吸器疾患研究部　*中田　光*，濱野　栄美
*新潟大学医歯学総合病院生命科学医療センター
独立行政法人国立病院機構東京病院　川辺　芳子，益田　公彦，水井　英明，有賀　昭之，倉島　篤行
東京医科歯科大学細胞治療センター　森尾　友宏，清水　則夫

はじめに

多剤耐性結核は，いったん感染すると治療がきわめて難しく，周囲への感染拡大の危険性もある。また，患者は長期の入院を余儀なくされており，確実な治療法として外科的肺切除を受けることが多い。さらに病変の拡がり，肺機能などから外科的治療を受けられない患者では治療自体が非常に困難になる。

活性化自己T細胞輸注法は国立がんセンター研究所の関根らにより開発された方法で，自己血中のT細胞を固相化抗CD3抗体とインターロイキン2（IL-2）の存在下で培養し，1000倍程度に増殖させた後，体内に戻すものである。現在までに，700症例以上の患者に対し，延べ7000回以上の投与が行われた。ホジキン病，肝細胞癌，卵巣腫瘍など様々な腫瘍で縮小効果を認めるとともに，慢性活動性エプスタンバールウイルス（EBV）感染症，免疫不全症に合併した化学療法抵抗性のサイトメガロウイルス（CMV）感染症，カリニ肺炎などに効果を認めている。副作用は感染症に対する反応としての発熱程度であることが多く，強い炎症が起きた場合もステロイド剤などでコントロールすることが可能であり，その安全性が東京医科歯科大学においても再確認された。このようにT細胞機能不全がある症例でもT細胞を生体外で活性化，増殖させ，生体内に戻すことによりT細胞機能を高めることが可能であることが明らかになってきている。

結核感染症に対する生体側の防御機構はT細胞を中心とする細胞性免疫が担っている。多剤耐性結核患者では細胞性免疫機能の低下が指摘されており，治療法のない多剤耐性結核患者に活性化自己T細胞輸注法の効果が期待される。活性化T細胞療法を薬剤抵抗性細菌感染症の新規治療法として確立できれば，長期隔離を余儀なくされている患者の退院が可能となり，患者にとって福音となるばかりでなく医療経済的にも重要な進歩となる。さらに，長期的には薬剤抵抗性細菌感染症の封じ込めに繋がり，社会に対して多大な貢献となるだろう。

対象および方法

（A）臨床試験（パイロットスタディー）

平成14年春に東京病院，東京医科歯科大学，国際医療センターからなるプロジェクトチームが結成され，免疫不全症に対する活性化T細胞輸注療法を参考にしつつ，多剤耐性結核患者に対する活性化T細胞輸注療法プロトコールを作成，平成14年10月に東京病院倫理委員会に申請・承認を得た。

その概要は，以下のとおりである。

①フェーズ：院内臨床試験（単施設）

②目的：多剤耐性結核患者を対象に，活性化T細胞輸注療法の安全性と有効性について調べる。

③対象：過去3カ月以上持続排菌している多剤耐性結核患者で，過去6カ月間治療レジメンの変更がない者。

症例1　喀痰塗抹陰性　培養持続陽性の患者

症例2　喀痰塗抹持続陽性の患者

症例3　喀痰塗抹持続陽性，巨大空洞をもつ患者

④用法・用量

試験投与：本治療の前に1/10量の活性化T細胞を点滴静注し，有害事象の発現を調べる。副作用がなければ，本投与を開始する。

本投与：

プロトコール1：10^9個の自己活性化Tリンパ球を2週間おきに計6回輸注

プロトコール2：10^9個の自己活性化Tリンパ球を4日おきに計3回輸注

2週間あけて同様の輸注を行う。

⑤プリマリーエンドポイントおよび観察項目

リンパ球輸注開始後，3カ月間，培養検査で喀痰中の菌陰性状態が持続するものを有効とする。治療後の有害事象の観察，およびCRP，血沈，ツベルクリン反応，末梢血early secreted antigenic target 6 kDa protein（ESAT-6）刺激下インターフェロンγ産生能（QuantiFERON-TB test）の変化をみる。

（B）CD3-AT法による活性化T細胞の作製

ヘパリン添加末梢血10 mlから比重遠心法にて単核球分離し，抗CD3抗体固相化フラスコ中でIL-2と共に7日間培養し，一部凍結保存し，患者へ投与日より16日前に再度解凍し，抗CD3抗体なしで2日間培養，その後抗CD3抗体固相化フラスコ中でIL-2と共に7日間培養し，ガス透過性バックに移し，抗CD3抗体なしで7日間培養し，細胞を生理食塩水で洗浄後，1% Human Serum Albumin（HSA）加生理食塩水に浮遊，点滴静注する。

（C）*In vitro* 患者活性化T細胞によるBCG殺菌能の検討

多剤耐性結核患者および健常者の末梢血単核球より抗CD3抗体＋IL-2刺激（anti CD3-activated T lymphocyte method: CD3-AT法）により活性化増幅したT細胞を，あらかじめBCG菌を感染させた自己単球由来マクロファージに添加し，殺菌能活性化に必要な細胞数の検討を行った。自己単球由来マクロファージ1×10^5/wellにMOI 1.0の割合でBCG菌を感染させ，2時間後，マクロファージを洗浄し，非感染菌を除去した。直後，1×10^5/wellの割合で自己活性化T細胞または非活性化リンパ球を添加し，4日間培養後，マクロファージ内BCG菌を0.1% Sodium Dodecyl Sulfate/Sodium Buffered Saline（SDS/PBS）で細胞溶解させ，7H10プレートに撒き，培養2～3週間後にコロニーカウントした。

結　果

最初に活性化T細胞がマクロファージによる抗酸菌殺菌能を増強させるかどうかを検討した。4人の多剤耐性結核患者および1人の健常者の単球由来マクロファージ内のBCG殺菌実験では，自己活性化T細胞を添加すると添加していないものに比べて$\log_{10}$で2オーダーの殺菌が観察された。非活性化自己リンパ球は全く効果がなかった（図1）。

CD3-AT法により活性化増幅したT細胞を，CD4陽性細胞とCD8陽性細胞に分離し，いずれの細胞が殺菌能を活性化するのかを検討したところ，CD4陽性，CD8陽性細胞ともに分離をしない活性化T細胞に比べて殺菌能は弱かった（data not shown）。このことから，多剤耐性結核患者に対しては，サブセットに分離せず，全T細胞を活性化したものを用いることに決定した。

次に患者に投与する活性化T細胞の表面活性化マーカーのFACS解析では，CD25は，90%以上，CD69は10%程度発現していたが，Interferon-γおよびtumor necrosis factor α（TNF-α）の産生は全くなく，抗CD3抗体による再刺激で産生するようになった。つまり，患者に静注する活性化T細胞はrestingの状態であることが確認された（図2）。

3例の多剤耐性結核患者をプロトコール1に従って治療した。3例ともに輸注中，輸注後に特記すべき有害事象は見られなかった。排菌量の変化であるが，症例1では治療前に喀痰培養持続陽性であったのが，治

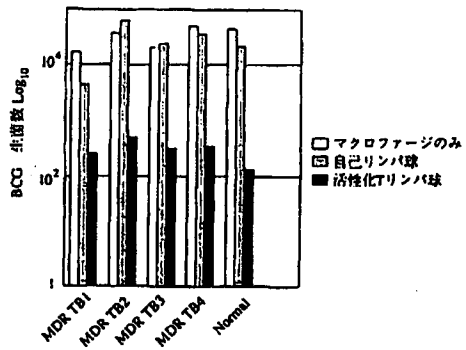

図1　活性化T細胞による自己単球由来マクロファージ内BCG殺菌
MDR: multidrug resistance

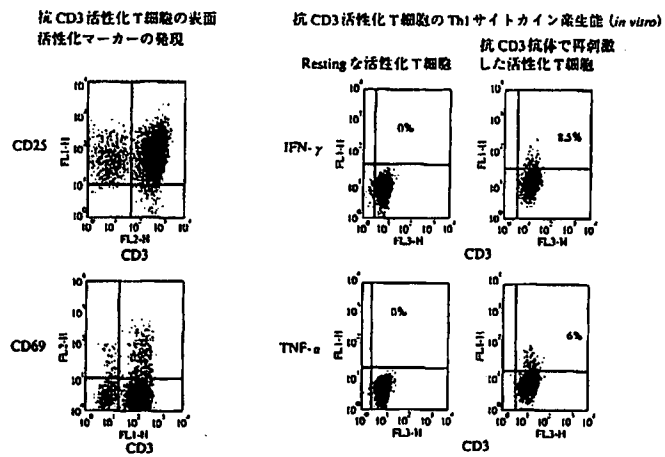

図2　活性化T細胞の表面マーカー発現とサイトカイン産生

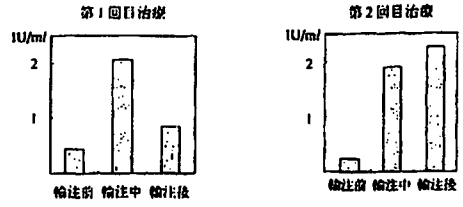

図3 症例2の Quantiferon TB ESAT-6 刺激後末梢血の IFN-γ 産生

療後3カ月間喀痰培養陰性となり，その後再度培養陽性となった。症例2では，喀痰塗抹培養陽性であったが，治療後喀痰塗抹・培養ともに陰性が治療後5カ月間持続し，その後培養陽性となった。症例3に関しては，全く効果なく，排菌量に変化がなかった。症例2については，プロトコール2による再治療を行った。治療後2カ月間培養陰性となったが，再度陽性となっている。

以上の結果をまとめると治療した3例のうち，2例が有効だったが，効果は一時的であった。注目すべきことに，有効であった2例において治療前後のツベルクリン反応（data not shown）および結核菌 ESAT-6 に対する末梢血のインターフェロンγ産生能が増強されたことである（図3）。

このように，活性化T細胞輸注により，減弱していた結核菌に対する遅延型アレルギー反応が賦活化された可能性がある。

考　察

本研究は，細胞療法の一種である活性化T細胞輸注療法を難治性の多剤耐性結核の治療へ応用し，化学療法，外科療法に加えて免疫療法も将来取りうる選択肢に加えることを目的としている。今回の検討は，あくまでパイロット的な投与であったが，それでも副作用が認められなかったこと，2例において短期間で一時的であったが，排菌が停止したことは今後の治療法の改良に希望がもてる。これまで，多剤耐性結核に対してはインターロイキン2やインターフェロンγ投与などにより免疫賦活を計る治療法はすでに試みられているが，顕著な効果はでていない。活性化T細胞療法による難治性感染症治療の研究：抗CD3抗体＋IL-2刺激（CD3-AT法）により活性化増幅したT細胞を利用した免疫療法は，がんの免疫治療法として開発され，その治療効果と安全性は無作為抽出試験により証明されている。この治療法のユニークな点は，末梢血T細胞すべてを活性化増幅して投与することであり，特異的T細胞を誘導して治療に用いる方法に比べて，細胞調製が容易であること，様々な様的に対する効果が期待できるなどの長所がある。

活性化T細胞には *in vitro* においてマクロファージ内のBCG菌殺菌能の増強効果が認められたが，輸注後に活性化T細胞は速やかに組織に移行するといわれており，輸注後のツベルクリン反応の増強や末梢血のESAT-6への反応性の増大は，輸注された活性化T細胞の直接的な作用というよりは，結核菌の慢性感染によるアネルギー状態が解除された結果と考えるほうがよいかもしれない。

今後は，寛解期間を延長するために活性化T細胞の輸注期間を延長したり，他の免疫療法例えば樹状細胞療法と組み合わせるなどの工夫により，本治療の実用化を目指していきたい。

The 79th Annual Meeting Symposium

STRATEGIES AGAINST MULTIDRUG-RESISTANT TUBERCULOSIS

Chairpersons: [1]Tetsuya TAKASHIMA and [2]Yoshiko KAWABE

Abstract Pulmonary tuberculosis can be cured by 6 months chemotherapy, consisting of isoniazid (INH), rifampicin (RFP), pyrazinamide (PZA), and etahanbutol (EB). However, the patients with pulmonary tuberculosis caused by multidrug-resistant tuberculosis (MDR-TB) bacilli, defined as resistance to at least INH and RFP, poorly respond to this regimen. Therefore, the epidemic of MDR-TB in the community is a major threat to tuberculosis control.

According to the interim report of the survey of drug-resistant tuberculosis carried out by Tuberculosis Research Committee Japan in 2002, the prevalence of MDR-TB among new cases, previously treated cases and combined cases was 0.9%, 9.9% and 2.1%, respectively. Thus, the latest Japanese prevalence of MDR-TB was as high as the median prevalence of 72 geographical settings in the world, reported in the WHO/IUATLD Global Project on Drug Resistance Tuberculosis Surveillance, 1994-1999. In Japan, there is still an estimated 2,000 cases of MDR-TB patients. In the last meeting of the Japanese Society for Tuberculosis, an outbreak of MDR-TB in tuberculosis wards was reported, and a careful infection control of MDR-TB was recognized again.

To work out the strategy for the elimination of MDR-TB, two issues were taken up in this symposium. First, not to make new MDR-TB cases, an intervention in the development and spread of MDR-TB was discussed. Second, the effectiveness of conventional anti-tuberculosis chemotherapy and pulmonary resection in the treatment of patients with MDR-TB was reevaluated, and a new approach for the treatment of chronic cases was also discussed.

Dr. Koji Sato (National Amamiwakouen Sanatorium) surveyed the number of patients with MDR-TB in 72 hospitals with tuberculosis wards, and examined the clinical characteristics of chronic cases who had been expecting MDR-TB bacilli in the sputum for more than 5 years. One hundred and twenty-one of 149 chronic cases (81%) in this study were sputum-smear positive. Thirty-seven of them (25%) were outpatients. Thus, the high risk of MDR-TB transmission and the difficulties of infection control of chronic cases were reported.

Dr. Yuka Sasaki (National Hospital Organizatinn Chiba East National Hospital) conducted the questionnaire survey to the ordinance-designated cities and National Sanatoria Hospitals in Japan. Only a few contacts of patients with MDR-TB received preventive treatment, mainly due to the difficulties of diagnosis of latent MDR-TB infections and no effective treatment regimens. She pointed out the importance of preventive treatment guideline for contacts of patients with MDR-TB.

Dr. Masako Wada (Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association) examined the details of acquired MDR-TB cases. Among 2,375 pulmonary tuberculosis patients newly treated in Fukujuji Hospital form 1991 to 2002, 4 cases had developed drug resistance to INH and RFP during treatment. First case was initially INH mono-resistant, which had treated with INH, RFP and EB. Second case had received a sequential mono-therapy after serious adverse reaction. The remaining two cases were supposed to be re-infected with MDR-TB during treatment. This study indicated the importance of improvement of treatment guideline for patients with adverse drug reactions and infection control of MDR-TB in the sanatoria hospitals, in addition to the avoidance of sequential mono-therapy.

Unlike the treatment of drug susceptible tuberculosis, it is not possible to develop a standard treatment regimen for MDR-TB. To know the treatment outcome by the number of susceptible drugs included in each regimen, Dr. Takayuki Nagai (Osaka Prefectural Medical Center for Respiratory and Allergic Diseases) reviewed the 37 patients with pulmonary MDR-TB, knowing the results of susceptibility testing to all 11 anti-tuberculosis drugs, such as INH, RFP, PZA, EB, streptomycin (SM), kanamycin (KM), Enviomycin (EVM), Ethionamide (TH), Cycloserine (CS), Para-aminosallcylic acid (PAS) and levofloxacin (LVFX). Among 11 patients who had received at least 3 susceptible drugs of PZA, LVFX and aminoglycoside, 10 patients (90.9%) had favorable response, converting their sputum cultures to negative at 2 months after the start of chemotherapy. He said that surgical interventions should be considered for any cases, which will not be effectively treated by the regimens including PZA, LVFX and aminoglycoside.

Dr. Yuzo Sagara (National Hospital Organization Tokyo National Hospital) reviewed the surgical outcome of 28 patients with pulmonary MDR-TB with sufficient follow up data. All 8 patients, whose lung lesions had been completely removed, had achieved sputum-culture conversion after surgery and in combination with adequate chemotherapy. Even among 20 patients who still have some lesions after surgery, 14 patients (70.0%) had negative results of sputum cultures. Thus, it is shown that surgical intervention is a major treatment approach to MDR-TB.

Finally, Dr. Koh Nakata (Niigata University Medical Dental Hospital) reported a clinical trial of activated autologous T lymphocytes transfusion to chronic cases. This immunotherapy was well tolerated by all 3 patients. Two patients had responded to this treatment and their sputum culture had become negative for 3-5 months. The host immune up-

regulation was proved by the tuberculin skin test conversion and the increment of IFN-γ production by peripheral blood in response to EAST-6 antigen. It was shown that activated T lymphocyte transfusion might be an effective treatment measure for some chronic cases, by enhancing the host anti-mycobacterial defense systems.

MDR-TB control strategies should be primarily aimed at preventing the emergence of new cases. The rational approach devised by each panelist in this symposium will be the first step to containing the further spread of MDR-TB.

1. Current status of patients with multidrug resistant tuberculosis (MDR-TB) in the long term in Japan: [1]Koji SATO (National Amamiwakouen Sanatorium), Masashi MORI (National Hospital Organization Tokyo National Hospital)

We surveyed the number of MDR-TB cases in Japan. Four hundreds and eighty-seven cases (4.8%) of 10,208 tuberculosis patients registered in 72 hospitals were MDR-TB. Of them, 149 cases (30.6%) had been expecting MDR-TB bacilli in sputum for a long time more than 5 years. We examined the clinical profiles of these so called chronics. There were 33 females and 116 males. Ninety-eight (65.8%) of them were more than 60 years old. Thirty-seven (24.8%) were out patients. Among 103 cases with the reports of chest X-ray examination, 76 cases (73.8%) had cavity formations. Of them, 24 cases (64.9%) were sputum-smear positive. Difficulties of management and treatment of chronics were recognized again.

2. Chemoprophylaxis for contacts of patients with multidrug-resistant tuberculosis: Yuka SASAKI (Department of Thoracic Disease, National Hospital Organization Chiba East National Hospital)

The chemoprophylaxis to the contacts of patients with multidrug-resistant tuberculosis was considered. The questionnaire survey was conducted to the ordinance-designated cities in Japan. Chemoprophylaxis was performed in 2.4% of contacts of patients with multidrug-resistant tuberculosis, and in the contacts, 20 cases were diagnosed as tuberculosis in the ordinance-designated cities for the past five years. Chemopruphylaxis to the contacts of patients with multidrug-resistant tuberculosis is not carried out positively from many problems in National Sanatoria Hospitals. The present condition is troubled by the correspondence to the contacts of patients with multidrug-resistant tuberculosis.

3. Retrospective examination of treatment failures in newly diagnosed cases, whose strain had acquired multidrug resistance in initial treatment: Masako WADA (Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association)

Not to make a new multidrug resistant tuberculosis case, what should we do for it? First of all, we should treat all new pulmonary tuberculosis cases with 6-month regimen using INH, RFP, EB and PZA, if pyrazinamid is not contraindicated. In this review, two cases of far-advanced multidrug resistant pulmonary tuberculosis patients were presented. One patient received left pneumectomy with chemotherapy of second line anti-tuberculosis drugs, and she had been cured after the completion of 24-month chemotherapy. The other patients died due to massive hemoptysis with chronic respiratory failure at the age of 30 years old. It was supposed the critical different subject to their fates was the timing of reference to a specialist for tuberculosis treatment.

Among 2,608 newly diagnosed pulmonary tuberculosis patients from 1 January 1991 to 31 December 2002, only 4 cases (0.15%) had treatment failures with the emergence of multidrug resistance. First case infected with INH resistant strain was treated with INH, RFP and EB without PZA. Second case complicated with tuberculous pyothorax was also treated with above three drugs regimen. Third case had suffered from a serious skin adverse reaction, and then she had a sequential mono-therapy. The remaining case was suspected to have re-infected with MDR-TB strain.

We should initially treat all pulmonary tuberculosis patients with four drugs regimen. When the treatment failure had occurred due to drug resistant strain, adverse drug reactions or other reasons, it is essential to consult with a specialist for tuberculosis treatment. It should be never done to add anti-tuberculosis drugs one by one to the case of treatment failure.

4. Treatment outcomes of multidrug-resistant tuberculosis: [1]Takayuki NAGAI, Tetsuya TAKASHIMA, Izuo TSUYUGUCHI (Osaka Prefectural Medical Center for Respiratory and Allergic Diseases)

[Objective] To study the results of anti-tuberculosis chemotherapy of the patients with diagnoses of MDR-TB in our hospital and determine the adequate chemotherapy regimen for MDR-TB.

[Methods] Retrospective study of 37 cases of MDR-TB patients in our hospital between 1999 and 2002. In this study, the five cases were excluded, because they had not received TB treatment for at least 6 months.

[Results] The sputum culture conversion rates at 6 months after starting chemotherapy were 68.8% (22/32). Of them, 2 patients had relapsed bacteriologically during 2 years chemotherapy, 1 patient died, and 2 patients never completed a satisfactory course of treatment. Success rate of treatment was 50.0% (16/32). When 4 or more susceptible drugs were used, treatment success rate was significantly higher than 3 or less drugs were used ($p=0.012$). Among 11 patients who had received at least 3 susceptible drugs of PZA, LVFX and aminoglycoside, 10 patients (90.9%) had favorable response, converting their sputum cultures to negative at 2 months after the start of chemotherapy.

[Conclusion] Surgical interventions should be considered for any cases, which will not be effectively treated by the regimens including PZA, LVFX and aminoglycoside.

5. Surgical management of multidrug resistant pulmonary tuberculosis (MDR-TB): Yuzo SAGARA (National Hospital Organization Tokyo National Hospital)

From January 1991 through December 2002, we operated on 36 MDR-TB patients. Eight complete resections, 23 incomplete resections, and 5 thoracoplasties were performed.

Final success rate of complete resection was 100%. On the other hands, that of incomplete resection was 70%. The cases of MDR-TB within four resistant drugs were successfully treated by incomplete resection.

Complete resection of the pulmonary lesion was the best surgical treatment for MDR-TB, if it is possible.

6. A clinical trial of activated autologous T lymphocytes transfusion for multidrug resistant tuberculosis: [1]Koh NAKATA*, Emi HAMANO (International Medical Center of Japan, *Bioscience Medical Research Center, Niigata Medical Dental Hospital), Yoshiko KAWABE, Kimihiko MASUDA, Hideaki NAGAI, Haruyuki ARIGA, Atsuyuki KURASHIMA (National Hospital Organization Tokyo National Hospital), Tomohiro MORIO, Norio SHIMIZU (Center for Cell Therapy, Tokyo Medical and Dental University, Medical Hospital)

In multidrug resistant tuberculosis (MDR-TB), T cell function is supposed to be attenuated against *M. tuberculosis*. It is rational to consider that chronic infection lead the host immune system to be anergy state against pathogeons. This study was performed to evaluate the efficacy of activated T lymphocyte transfusion on MDR-TB to reactivate host defense system. One thousand million activated autologous T lymphocytes were transfused every two weeks to three patients with MDR-TB who were chronically positive AFB in their sputum. Two cases responded to this treatment and become negative bacilli in the sputum for 3–5 months, however, they reoccurred afterwards. The other one case did not respond at all. In all three cases, no side effect was observed. Interestingly, in two cases with response, tuberculin skin test and peripheral blood interferon gamma production reacting tuberculosis specific antigen, ESAT-6 were both dramatically augmented during negative bacilli in their sputa. Activated T cell transfusion is safe and may improve the anergy state in some patients with MDR-TB.

Key words: MDR-TB, Case management, Chemoprophylaxis, Treatment

[1]Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, [2]National Hospital Organization Tokyo National Hospital

Correspondence to: Tetsuya Takashima, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3–7–1, Habikino, Habikino-shi, Osaka 583–8588 Japan.
(E-mail: tetsuya@hbk.pref.osaka.jp)