(1×10^6/ml) prepared from mice 10 days after immunization, were stimulated with 10 μg/ml OVA or 10 μg/ml TRP-2. After the stimulation, IFN-γ- and IL-4-producing cells were examined by intracellular staining according to previously described methods.[22,24] In brief, 2 μM of monensin (BD Biosciences Pharmingen) was added for the last 4 hr of the culture. The cells were harvested and stained with 7-amino-actinomycin D and anti-CD4 (GK1·5)- or anti-CD8- allophycocyanin (BD Biosciences Pharmingen). Then, the cells were washed in 0·05% azide–1%FCS–phosphate-buffered saline, fixed with 1·6% formaldehyde, made permeable with 0·1% saponin and stained with anti-IFN-γ-FITC (XMG1.2) (BD Biosciences Pharmingen) and anti-IL-4-PE (11B11) (BD Biosciences Pharmingen) or isotype control antibodies. Stained cells were gated on live $CD4^+$ or $CD8^+$ cells and analysed by FACSCalibur. The amounts of IFN-γ and IL-4 in the culture supernatant after OVA or TRP-2 stimulation *in vitro* were quantified by ELISA following the manufacturer's instructions. The mAbs specific for mouse IFN-γ and IL-4 that were used for capture and detection of cytokines were purchased from BD Biosciences Pharmingen. ELISA of IL-12p40 was conducted using a murine IL-12 p40 OptEIA™ ELISA kit (BD Biosciences Pharmingen).

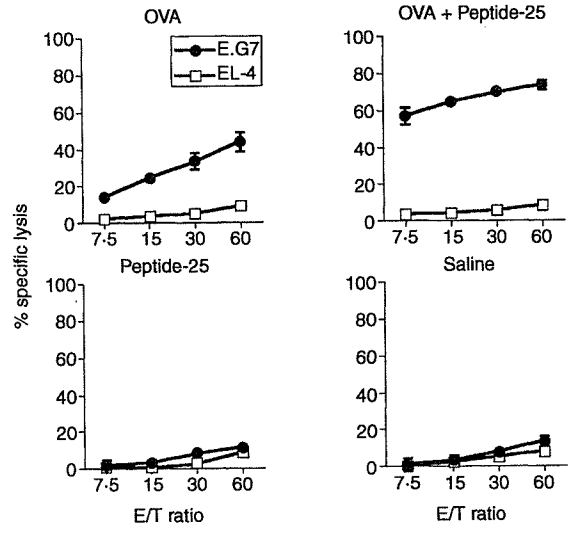
ELISA for anti-OVA antibody titration

For assessment of anti-OVA IgG1 and IgG2a levels, serum was collected from the immunized mice at 10 days after immunization and added to the OVA-coated plate. Biotinylated goat anti-mouse IgG1 antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) or biotinylated goat anti-mouse IgG2a (5.7.2) was applied and detection was performed using streptavidin-peroxidase (Zymed Laboratories Inc., San Francisco, CA).

Results

Peptide-25 enhances the generation of OVA-specific $CD8^+$ CTL response

Peptide-25, a 15-mer peptide of Ag85B is a major T-cell epitope recognized by $CD4^+$ I-A^b-restricted Th1 cells specific for Ag85B of *M. tuberculosis*.[20,21] Immunization of C57BL/6 mice with Peptide-25 induced the generation of IFN-γ- and TNF-α-producing Th1 cells that preferentially express TCRVβ11.[21] As $CD4^+$ Th1 cells can augment the $CD8^+$ CTL response[28,29] we examined whether immunization with a mixture of OVA and Peptide-25 can enhance the generation of an OVA-specific CTL response compared to OVA immunization. Three groups of mice were immunized with OVA in IFA, Peptide-25 in IFA, or a mixture of OVA and Peptide-25 in IFA. As a control, a group of mice was treated with IFA. Ten days after the immunization, spleen cells from each group of mice were



Lytic unit (/10^6)	OVA	OVA + Peptide-25	Peptide-25	Saline
E.G7	1·0	29·1	<0·01	<0·01
EL-4	<0·01	<0·01	<0·01	<0·01

Figure 1. Enhanced induction of OVA-specific $CD8^+$ CTL response in spleen by coimmunization with OVA and Peptide-25. Three groups of mice were immunized with OVA (10 μg) in IFA, OVA (10 μg) and Peptide-25 (10 μg) in IFA or Peptide-25 (10 μg) in IFA subcutaneously. Spleen cells from each group of mice 10 days after the immunization were subjected to OVA-specific CTL assay. CTL assay was conducted by incubating sensitized cells with ^{51}Cr-labelled E.G7 or ^{51}Cr-labelled EL-4 cells of various effector to target ratios at 37° for 4 hr. The values represent the geometric means and standard deviation of percentage specific lysis in triplicate cultures. Background lysis of the target cells was less than 10% of maximum ^{51}Cr-release. The number of LU was calculated from the dose–response curve obtained for each group. A representative result of a series of five experiments is shown.

stimulated *in vitro* for 5 days with heavily irradiated E.G7 as stimulator cells that express OVA linked to the MHC class I molecule. The responding cells recovered after the culture were subjected to OVA-specific CTL assay as effector cells. The CTL activity was assessed on a 4-hr ^{51}Cr-release assay using ^{51}Cr-labelled E.G7 or EL-4. Results clearly revealed that the spleen cells from OVA-immunized mice mounted a significant CTL response to E.G7, but not to EL-4 upon *in vitro* stimulation with irradiated E.G7 (Fig. 1). Interestingly, a robust OVA-specific CTL response was induced in the culture of the spleen cells from mice immunized with a mixture of OVA and Peptide-25 in IFA upon E.G7 stimulation. The enhancement of the OVA-specific CTL response by coimmunization of OVA with Peptide-25 was quantitatively confirmed by calculating the lytic unit. Spleen cells from Peptide-25-immunized mice or from IFA-treated mice did not mount a significant CTL response to E.G7 upon E.G7 re-stimulation *in vitro*, indicating that Peptide-25 immunization does not induce a polyclonal CTL response.

We confirmed that an enhanced OVA-specific CTL response was observed after coimmunization with Peptide-25 and MHC class I-binding OVA peptide (data not shown). The OVA-specific CTL activity in effector cells was abrogated completely by the depletion of $CD8^+$ T cells using anti-CD8 mAb plus complement treatment before CTL assay, while the CTL activity remained the same in the treatment of spleen cells with anti-CD4 mAb plus complement (data not shown).

To examine the enhancing effect of I-A^b-binding peptides other than Peptide-25 on OVA-specific CTL generation, three groups of mice were immunized with OVA in IFA, a mixture of OVA and Peptide-25 in IFA, or a mixture of OVA and Peptide-9 of Ag85B in IFA. As a control, a group of mice was immunized with OVA and Peptide-18 (non-I-A^b-binding peptide of Ag85B) in IFA. The generation of an OVA-specific CTL response in spleen cells was assessed 10 days after the immunization. While immunization with OVA and Peptide-25 induced a potent OVA-specific CTL response, the CTL response observed in spleens from mice immunized with OVA and Peptide-9 was much less, if present at all (data not shown). Immunization with a mixture of OVA and Peptide-18 did not show enhancement of the OVA-specific CTL response.

In separate experiments, we analysed the augmenting effect of Peptide-25 on the CTL response specific for TRP-2 peptide, which is an MHC class I-binding peptide of murine melanoma. We immunized C57BL/6 mice with TRP-2 in IFA or a mixture of TRP-2 and Peptide-25 in IFA twice with a 10-day interval. Spleen cells from each group of mice were stimulated *in vitro* with TRP-2 for 5 days and TRP-2-specific CTL assay was conducted. Co-immunization with TRP-2 and Peptide-25 induced a significant $CD8^+$ CTL response in T cells to TRP-2 and IFN-γ production, while TRP-2 immunization was ineffective (Fig. 2a,b).

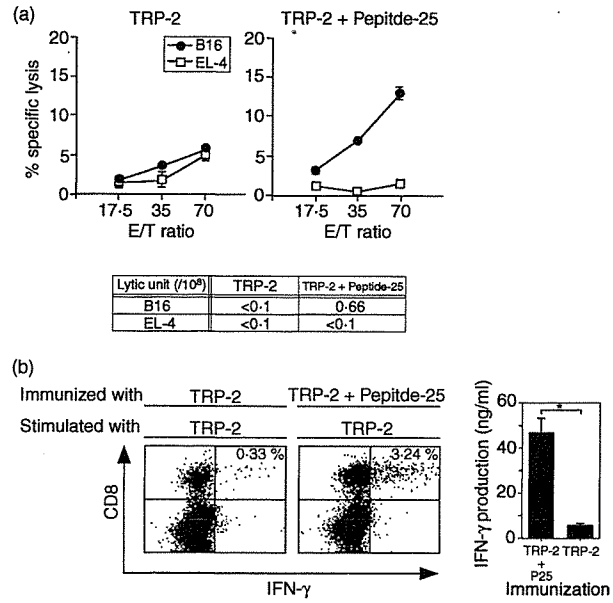


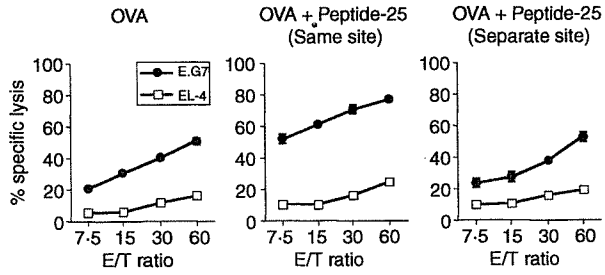
Figure 2. Enhancing effect of Peptide-25 on TRP-2-specific $CD8^+$ CTL response by coimmunization with TRP-2. (a) Two groups of mice were immunized subcutaneously with TRP-2 (10 μg) in IFA or TRP-2 (10 μg) and Peptide-25 (P25) (10 μg) in IFA. Spleen cells from each group of mice were subjected to TRP-2-specific CTL assay 10 days after the immunization. CTL assay was conducted by incubating sensitized cells with ^{51}Cr-labelled B16 melanoma or ^{51}Cr-labelled EL-4 cells of various effector to target ratios at 37° for 4 hr. The values represent the geometric means and standard deviation of percentage specific lysis in triplicate cultures. Background lysis of the target cells was less than 9% of maximum ^{51}Cr-release. The number of LU was calculated from the dose–response curve obtained with each group. A representative result of a series of three experiments is shown. (b) Ten days after immunization, spleen cells were stimulated with TRP-2 (10 μg/ml) for 2 days. Intracellular staining of IFN-γ was carried out on the recovered cells and they were examined by FACSCalibur. The percentages of IFN-γ-producing $CD8^+$ cells are presented in the upper right region. IFN-γ production of spleen cells in the culture supernatants was quantified by ELISA. The values represent the mean and standard deviation of the triplicate cultures. $^*P < 0{\cdot}01$ by Student's *t*-test.

Co-immunization of a mixture of OVA and Peptide-25 at the same site is required for the enhanced $CD8^+$ CTL response

We examined whether enhanced OVA-specific CTL generation by coimmunization with Peptide-25 can be induced when OVA and Peptide-25 are immunized separately. A group of mice was immunized with a mixture of OVA and Peptide-25 in IFA subcutaneously at the same site on the right-hand side of the abdomen. A group of mice was immunized with OVA in IFA and Peptide-25 in IFA separately (left and right sides of the abdomen, respectively). The CTL assay was conducted using spleen cells from each group of mice 10 days after the immunization. As shown in Fig. 3, the enhancement of the OVA-specific CTL response by Peptide-25 was observed only when a mixture of OVA and Peptide-25 in IFA was immunized at the same site. These results suggest that OVA and Peptide-25 need to be presented by the same antigen-presenting cells (APCs) for antigen processing to occur.

The enhancement of the OVA-specific cytolytic T-cell response by Peptide-25 depends on $CD4^+$ T cells and IFN-γ

To understand the efficacy of Peptide-25 to enhance OVA-specific cytotoxic activity, we examined whether IFN-γ-producing $CD4^+$ T cells contribute to the above enhancing effect of Peptide-25 on OVA-specific CTL generation. First, we depleted $CD4^+$ T cells *in vivo* by administering anti-CD4 mAb (GK1.5) to two groups of mice as



Lytic unit (/10^6)	OVA	OVA + Peptide-25 (Same site)	OVA + Peptide-25 (Separate site)
E.G7	1·8	15·1	1·7
EL-4	<0·01	<0·01	<0·01

Figure 3. Enhanced OVA-specific CD8$^+$ T-cell response induced by coimmunization with OVA and Peptide-25 at the same site. A group of mice was immunized with OVA (10 μg) and Peptide-25 (10 μg) in IFA at the same site subcutaneously. Another group of mice was immunized with OVA (10 μg) in IFA and Peptide-25 (10 μg) in IFA at two distant sites (separate sites). As a control, we also immunized a group of mice with OVA (10 μg) in IFA. Spleen cells from each group of mice 10 days after the immunization were subjected to OVA-specific CTL assay. CTL assay was conducted by incubating sensitized cells with ^{51}Cr-labelled E.G7 or ^{51}Cr-labelled EL-4 cells at various effector to target ratios at 37° for 4 hr. The values represent the geometric means and standard deviation of percentage specific lysis in triplicate cultures. Background lysis of the target cells was less than 10% of maximum ^{51}Cr-release. The number of LU was calculated from the dose–response curve obtained with each group. A representative result of a series of three experiments is shown.

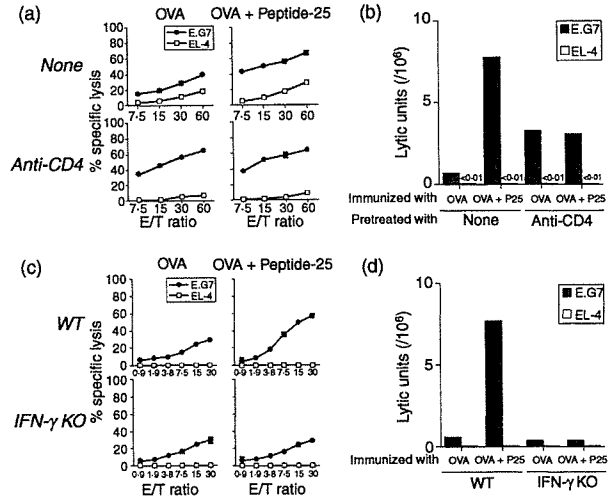


Figure 4. Role of CD4$^+$ T cells and IFN-γ in the enhancing effect of Peptide-25 on CTL response. (a,b) Two groups of wild-type mice were injected with anti-CD4 mAb (GK1.5) on days −13, −12, −11, −6, −5, −4, +1, +2 and +3 relative to immunization. Each group of mice was immunized with OVA (10 μg) in IFA or a mixture of OVA (10 μg) and Peptide-25 (10 μg) in IFA on Day 0. As controls, two other groups of mice had been treated with normal rat IgG in place of GK1.5 and immunized with OVA in IFA or a mixture of OVA and Peptide-25 in IFA. Spleen cells from each group of mice were subjected to *in vitro* OVA-specific CTL assay 10 days after the immunization. (c,d). Either wild-type or IFN-γ$^{-/-}$ mice with C57BL/6 background were immunized with OVA (10 μg) in IFA or a mixture of OVA (10 μg) and Peptide-25 (P25) (10 μg) in IFA. Spleen cells from each group of mice were subjected to OVA-specific CTL assay 10 days after the immunization. CTL assay (a,c) and LU calculations (b,d) were conducted as described in Figure 1.

described in the Materials and methods. As a control, rat IgG was administered in place of anti-CD4 to another two groups of mice. All groups of mice were immunized with OVA in IFA or with a mixture of OVA and Peptide-25 in IFA. As shown in Fig. 4(a,b), an enhanced CTL response specific for OVA, mounted in a culture of spleen cells taken from mice immunized with a mixture of OVA and Peptide-25 in IFA, was abrogated by the anti-CD4 mAb treatment before immunization, and the level of CTL response, measured as LU, was similar to that mounted in spleen cells from OVA-immunized mice. Unexpectedly, an enhanced OVA-specific CTL response, mounted in a culture of spleen cells from OVA-immunized mice, was observed when anti-CD4 mAb mice had been treated before OVA immunization.

To examine the roles of IFN-γ-producing cells in the CTL response, we immunized both wild-type and IFN-γ$^{-/-}$ mice with a mixture of OVA and Peptide-25 in IFA. Spleen cells from each group of mice were then subjected to the *in vitro* CTL assay specific for OVA. The results revealed that an enhanced CTL response specific for OVA was mounted in spleen cells from wild-type mice immunized with a mixture of OVA and Peptide-25 in IFA, while the enhancement was not observed in spleen cells from IFN-γ$^{-/-}$ mice (Fig. 4c,d). These results indicate that Peptide-25-reactive IFN-γ-producing T cells contribute to the enhancing effect of Peptide-25 on OVA-specific CTL generation.

Co-immunization with a mixture of OVA and Peptide-25 increases proportions of OVA-specific CTL precursors

To examine the cellular mechanisms of the enhancing effect of coimmunization of mice with a mixture of OVA and Peptide-25 on CTL generation following *in vitro* OVA stimulation, we examined the frequencies of OVA-specific CTL precursors. Spleen cells from mice were immunized with either OVA in IFA or a mixture of OVA and Peptide-25 in IFA 10 days before the experiments. The cells were then stained with OVA peptide-loaded H-2K^b:Ig protein and anti-mouse IgG1–PE and analysed using FACSCalibur. The results revealed that frequencies for OVA-specific CTL precursors in spleen cells (0·42%) from mice immunized with a mixture of OVA and Peptide-25 were about two-fold higher than those in spleen cells (0·21%) from OVA-immunized mice (Fig. 5).

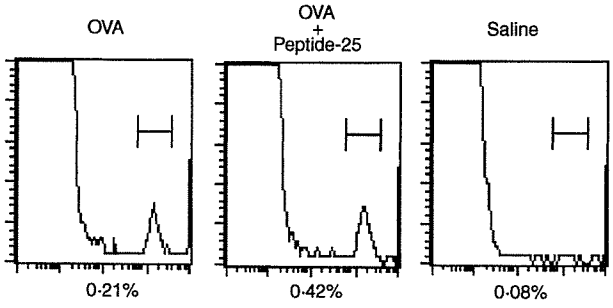


Figure 5. Frequency analysis of OVA-specific CTL. Two groups of mice were immunized with either OVA in IFA or OVA and Peptide-25 in IFA. Spleen cells from each group of mice were prepared 10 days after the immunization and stained with 4 μg of OVA peptide (SIINFEKL)-loaded H-2K^b:Ig protein and incubated for 60 min at 4°. After washing with staining buffer, cells were resuspended in 100 μl staining buffer containing appropriately diluted anti-mouse IgG1-PE and anti-CD8-FITC. After washing twice with staining buffer, cells were analysed using FACSCalibur.

Co-immunization of mice with a mixture of OVA and Peptide-25 can suppress E.G7 growth, leading to tumour rejection *in vivo*

We examined whether the immunization of C57BL/6 mice with a mixture of OVA and Peptide-25 was effective as a prophylactic intervention into the growth of E.G7. Three groups of 12 mice each were immunized with OVA in IFA, Peptide-25 in IFA, or a mixture of OVA and Peptide-25 in IFA. As a control, a fourth group of 12 mice was injected with IFA. Ten days after the immunization, we transplanted viable E.G7 tumour cells onto the backs of the mice and monitored tumour growth (Fig. 6a) and survival (Fig. 6b) after tumour challenge. As shown in the figures, the transplanted E.G7 established tumour masses within days of the tumour challenge in the abdomen of both the control group and the Peptide-25-immunized group of mice, leading to the death of all animals by day 30. Tumour growth in mice immunized with OVA in IFA was slightly delayed compared with that in the control mice however, all mice died by day 40 after the tumour challenge. Interestingly, mice immunized with a mixture of OVA and Peptide-25 in IFA showed significantly delayed tumour progression. Among these 12 mice, the E.G7 tumours of six of them were eradicated by Day 60 after the tumour challenge and the lifetimes of the mice were continued, tumour-free, for up to 100 days. By contrast, all mice in the other groups died within 40 days after the tumour challenge. These results indicate that coimmunization of Peptide-25 with OVA enhances not only an OVA-specific CTL response *in vitro* but also induces a potent antitumour immunity against OVA-expressing tumour cells *in vivo*.

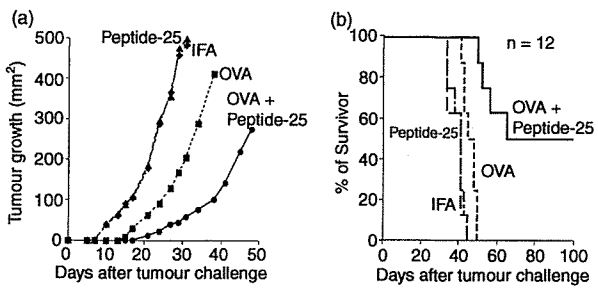


Figure 6. Enhancement of antitumour immunity by Peptide-25. (a) Suppression of E.G7 growth by augmented induction of E.G7-specific immunity. Three groups of mice were immunized with OVA (10 μg) in IFA, OVA (10 μg) and Peptide-25 (10 μg) in IFA or Peptide-25 (10 μg) in IFA subcutaneously. As a control, a group of mice was injected with IFA. All groups of mice were challenged with 5×10^5 viable E.G7 cells subcutaneously 10 days after the immunization. Growth of E.G7 tumour was monitored by measuring its size periodically (2- to 3-day intervals) and expressed as mm^2. (b) Survival of E.G7-bearing mice. The percentages of survivors in the respective groups shown in (a) are displayed.

The enhancement of the Th1 response to OVA by coimmunization with Peptide-25

To understand the mechanisms of the enhancing effect of Peptide-25 on the OVA-specific CTL response, we examined the Th1 generation specific for OVA when C57BL/6 mice were immunized with OVA in IFA, Peptide-25 in IFA, or a mixture of OVA and Peptide-25 in IFA. First, we measured anti-OVA antibody in the serum of each group of mice by ELISA 10 days after immunization. As shown in Fig. 7(a), OVA-immunized mice produced predominantly anti-OVA IgG1 antibody and produced lower levels of the anti-OVA IgG2a antibody. In contrast, mice immunized with a mixture of OVA and Peptide-25 produced predominantly anti-OVA IgG2a antibody. We could not detect any anti-OVA antibody in sera from Peptide-25-immunized mice (Fig. 7a).

To evaluate the cytokine-producing profiles in CD4$^+$ T cells in the spleen, spleen cells from each group of immunized mice were stimulated *in vitro* with OVA for 48 hr, and the proportions of IFN-γ- and IL-4-producing cells were examined by intracellular cytokine staining. Cells stained were gated on live CD4$^+$ cells and analysed by FACS. The results revealed that we could detect IFN-γ-producing CD4$^+$ T cells but not IL-4-producing cells when spleen cells from mice immunized with a mixture of OVA and Peptide-25 were stimulated with OVA (Fig. 7b). In contrast, OVA stimulation of spleen cells from OVA-immunized mice could become both IFN-γ- and IL-4-producing cells. We confirmed the enhancement of OVA-induced IFN-γ production by ELISA (Fig. 7b). Co-immunization of OVA with Peptide-9 slightly enhanced the proportions

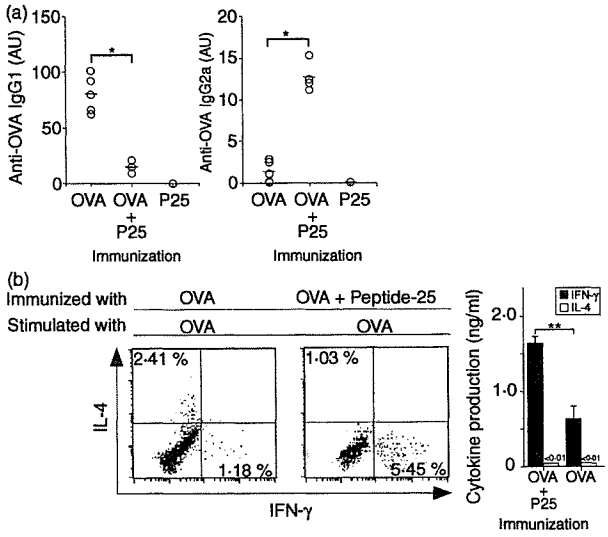


Figure 7. Enhancement of OVA-specific Th1 response by coimmunization of C57BL/6 mice with OVA and Peptide-25. Two different groups of mice were immunized with OVA (10 μg) in IFA, OVA (10 μg) and Peptide-25 (P25) (10 μg) in IFA, or Peptide-25 (10 μg) in IFA subcutaneously. (a) Serum anti-OVA IgG1 and IgG2a were titrated by ELISA 10 days after the immunization. Each open circle represents the results of an individual mouse. The horizontal bar represents the mean value of six mice. $*P < 0{\cdot}01$ by Student's *t*-test. (b) Ten days after immunization, spleen cells were stimulated *in vitro* with OVA (10 μg/ml) for 4 days. Intracellular staining of IL-4 and IFN-γ was carried out to the recovered cells. Cells stained were gated on live $CD4^+$ cells and examined by FACSCalibur. The percentages of IL-4- and IFN-γ-producing $CD4^+$ T cells are presented in the upper left and lower right regions, respectively. IL-4 and IFN-γ produced in the culture supernatants were titrated by ELISA. The values represent the mean and standard deviation of the triplicate cultures. $**P < 0{\cdot}05$ by Student's *t*-test.

of IFN-γ-producing cells, while coimmunization with Peptide-18 did not (data not shown). We did not observe differences in proportion of $CD4^+$ $CD25^+$ T cells between Peptide-25-immunized and Peptide-9-immunized T cells. These results suggest that the weak Th1-inducing ability of Peptide-9 correlates with a weak ability to enhance OVA-specific CTL generation when coimmunized with OVA.

The involvement of Peptide-25-reactive T cells in the induction of an OVA-reactive Th1 response was examined by using the rat anti-mouse clonotypic TCR mAb KN7 that can recognize TCR expressed on the Peptide-25-reactive Th1 clone of C57BL/6 mice. Enhanced induction of an OVA-specific IFN-γ-producing T-cell response in mice immunized with a mixture of OVA and Peptide-25 was impaired when we injected KN7 intraperitoneally 1 day before the immunization. In contrast, the control group that received rat IgG injection in place of KN7 did not show this suppressive effect (data not shown).

Peptide-25-reactive T cells can activate DCs through Peptide-25 leading to enhanced induction of OVA-presenting activity

The enhancement of an OVA-specific CTL response by coimmunization of Peptide-25 with OVA may be because the Peptide-25 directly or indirectly affects the OVA-presenting activity of APCs. To investigate this issue, we first evaluated the antigen-presenting activity of DCs. We propagated immature DCs *in vitro* by culturing bone marrow cells with GM-CSF and IL-3 for 6 days. They were stimulated for 48 hr with Peptide-25 in the presence of splenic $CD4^+$ T cells from P25 TCR-Tg mice. As a control, DCs were also stimulated with lipopolysaccharide. The expression of surface markers such as CD40, CD80, CD86, MHC class I antigen and intracellular adhesion molecule-1 (ICAM-1) was analysed using FACSCalibur. The results revealed that Peptide-25 stimulation alone did not alter the expression of the above surface markers on DCs (data not shown). When we cocultured DCs with $CD4^+$ T cells from P25 TCR-Tg mice together with Peptide-25, the expressions of MHC class I and ICAM-1 were enhanced to a similar extent to that found under lipopolysaccharide stimulation and this led to the induction of IL-12p40 production (Fig. 8a). Such DCs showed more effective OVA presentation to $CD8^+$ T cells from OT-1 mice and enhanced OT-1 cell divisions (Fig. 8b). These results suggest that Peptide-25-reactive $CD4^+$ T cells directly activate DCs in the presence of Peptide-25, leading to effective OVA cross-presentation for the activation of $CD8^+$ T cells.

Discussion

Antitumour immune responses involve complex interactions among various immunocompetent cells. $CD8^+$ CTLs are major effector cells capable of direct tumour destruction both *in vivo* and *in vitro*, and they recognize MHC class I binding peptides derived from molecules with altered expression in tumour cells.[28–30] Although the need for $CD4^+$ Th cells in regulating $CD8^+$ T cells has been documented, their target epitopes and functional impact in antitumour responses remain unclear.

There has been a recent reappraisal of the role and importance of $CD4^+$ Th cells in antitumour responses. $CD4^+$ Th cells are considered to contribute to the activation of $CD8^+$ CTLs through the expression of the CD40 ligand and production of cytokines, such as IL-2 and IFN-γ that are essential for the proliferation and maturation of $CD8^+$ CTL precursors. $CD4^+$ Th cells recognize MHC class II binding peptides on APCs and their interaction may result not only in the activation and priming of $CD4^+$ Th cells, but also in the activation of APCs themselves.[31–33] Consequent to these mutual activations, APCs prime and activate $CD8^+$ CTLs specific for the

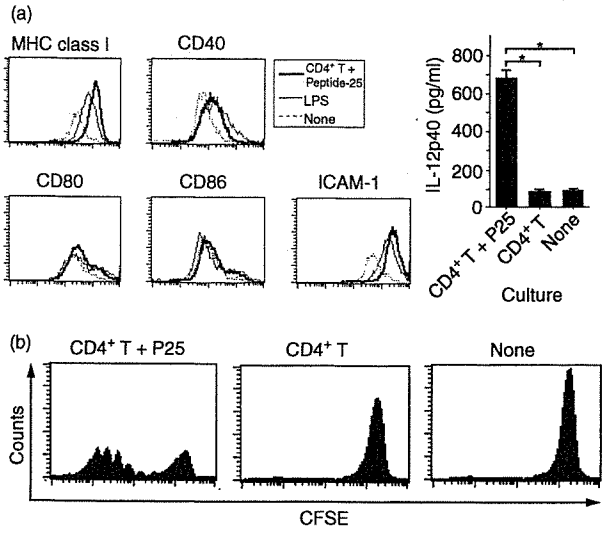


Figure 8. Activation of DCs by culturing with Peptide-25 in the presence of $CD4^+$ T cells from P25 TCR-Tg mice. (a) Immature DCs were propagated by culturing bone marrow cells with GM-CSF (20 ng/ml) and IL-3 (20 ng/ml) for 6 days. The cells recovered (5×10^5) were cultured with lipopolysaccharide (LPS; 5 μg/ml), Peptide-25 together with $CD4^+$ T cells (5×10^5) from P25 TCR-Tg mice or left untreated for 48 hr. The expression of surface markers was assessed by FACS analysis. IL-12p40 in the cultured supernatant was assessed by ELISA. (b) CFSE-labelled $CD8^+$ T cells (5×10^5) from OT-1 mice were cultured with immature DCs (5×10^5) and OVA (10 μg/ml) for 4 days. The cells were cocultured with $CD4^+$ T cells (5×10^5) from P25 TCR-Tg mice and Peptide-25 (P25) (10 μg/ml) (left panel) or $CD4^+$ T cells from P25 TCR-Tg mice (middle panel). Subsequently, cell division of the $CD8^+$ T cells was monitored by FACSCalibur.

tumour antigen or peptides. This scenario of cellular interaction assumes that $CD4^+$ Th cells and $CD8^+$ CTLs may not necessarily be in direct or close association. $CD4^+$ T cells are also implicated in the activation of tumoricidal macrophages that are involved in tumour clearance. Cytokines produced by $CD4^+$ T cells can recruit and activate macrophages and eosinophils, linking the T-cell response with the innate immune response.

In this study using OVA as neo-tumour antigen in a mouse model, we investigated the contribution of the Th1 epitope within the 15 amino acid residues of the Ag85B protein. We then examined the significance of the defined Th1 epitope regarding $CD8^+$ CTL generation and tumour eradication. We demonstrated that the coimmunization of Peptide-25 and the OVA CTL epitope resulted in a marked increase in the OVA-specific $CD8^+$ CTL response (Fig. 1). The selection of immunization site for Peptide-25 and OVA is important and both should be immunized at the same site (Fig. 3). The augmenting effect of Peptide-25 on $CD8^+$ CTL generation was cancelled by *in vivo* administration of the anti-CD4 mAb and was diminished in IFN-$\gamma^{-/-}$ mice (Fig. 4), indicating that the $CD4^+$ T cells and IFN-γ-producing T cells are required. It remains elusive why the enhancement of OVA-specific CTL activity was abrogated when IFN-$\gamma^{-/-}$ mice were immunized with the mixture of Peptide-25 and OVA. As we reported, the generation of Peptide-25-reactive $CD4^+$ T cells significantly decreases in IFN-$\gamma^{-/-}$ mice as compared with wild-type mice. Furthermore, Peptide-25 stimulation of Peptide-25-immunized cells induces IL-2 production that is also impaired in IFN-$\gamma^{-/-}$ cells.[22] IFN-γ-dependent IL-2 production may be indispensable to enhance the OVA-specific $CD8^+$ CTL generation and expansion in this particular system. Whilst we favour the possibility that impaired production of IFN-γ by $CD4^+$ T cells contributes to the reduction of CTL activity seen in mice primed with Peptide-25 and OVA we cannot exclude the possibility that ablation of IFN-γ production by $CD8^+$ T cells also plays a role.

Another explanation is that IFN-γ may play an important role in a cell-to-cell contact between Peptide-25-specific $CD4^+$ Th cells and APCs to enhance the induction of the OVA-specific $CD8^+$ CTL response. APCs are capable of processing and presenting exogenous antigens along with MHC class I molecules to $CD8^+$ T cells, which is termed antigen cross-presentation. The antigenic peptide generation for cross-presentation appears to be dependent on both the ubiquitin–proteasome system and the transporter associated with antigen processing.[34–37] Imai and his colleagues recently demonstrated that exogenously added OVA is accumulated in microsomal fractions, including the endoplasmic reticulum and late endosomes followed by retrograde transport to the cytoplasm through the Sec61 transporter complexes.[38] IFN-γ may facilitate these processes of OVA cross-presentation by APCs leading to enhancement of the induction of the OVA-specific $CD8^+$ CTL response.

Unexpectedly, our result showed that anti-CD4 mAb treatment before OVA immunization in mice enhanced the OVA-specific CTL response. Although we do not have concrete evidence, anti-CD4 mAb treatment may eliminate OVA-specific $CD4^+$ T cells that negatively regulate OVA-specific $CD8^+$ CTL generation.

Among I-A^b-binding peptides, Peptide-25 showed the most potent effect that correlated with potency for the *in vivo* induction of Th1 generation to each peptide. These results indicate that a Th1 epitope, regardless of the difference in its molecular origin, exhibits a helper activity for *in vivo* $CD8^+$ CTL generation. It is unclear why I-A^b binding Peptide-9 does not augment the induction of the OVA-specific CTL response. Peptide-9 showed lower immunogenicity for Th1 induction specific for its own compared with Peptide-25.[22] We analysed the Foxp3 expression in Peptide-9-reactive T cells by FACS and found no significant increase in the $Foxp3^+$ T regulatory cell population compared with Peptide-25-reactive T cells (data not shown). These results imply that the weak

potency of Peptide-9 to augment the OVA-specific CTL response is well correlated with its weak Th1-inducing potency instead of a T regulatory cell induction.

Both quantitative and qualitative changes may be involved in the Th effect of Peptide-25 in the CTL response. We think that a two-fold increase in the frequency of OVA-specific $CD8^+$ T cells and an increase of the lytic unit are qualitatively correlated with the Th effect of Peptide-25 (Figs 1–4). In addition, Peptide-25 stimulation of spleen cells from Peptide-25-immunized wild-type mice induces secretion of IL-2 that is impaired in IFN-$\gamma^{-/-}$ mice.[22] IL-2 produced by the Th1 cells may also enhance and expand the CTL maturation quantitatively. To evaluate qualitative changes more directly, we examined Granzyme B expression in OVA-reactive $CD8^+$ T cells. Results revealed that we did not observe significant increases in Granzyme B-expressing cells in the OVA-stimulated $CD8^+$ T cells in the presence of Peptide-25-primed Th cells compared with the precursors induced in the absence of the primed Th cells (data not shown). These results suggest that OVA-specific $CD8^+$ T-cell precursors induced in the presence of primed Th cells may differ quantitatively from these induced in the absence of primed Th cells.

The basis for the strong 'helper' function of Peptide-25 in the $CD8^+$ CTL response to E.G7 rejection is unknown. There are several possibilities to account for the enhancing effect of Peptide-25 on Th1 and CTL generation specific for OVA when Peptide-25 and OVA were coimmunized at the same site. Peptide-25 may directly activate APCs through unknown molecules resulting in effective cross-presentation of OVA molecules to both Th1 and CTL precursors. To address this issue, we propagated bone-marrow-derived DCs *in vitro*, stimulated them with Peptide-25 and analysed the surface expression of activation markers on DCs. The results revealed that expression of MHC class I, ICAM-1, CD40, CD80 and CD86 was not enhanced upon Peptide-25 stimulation (data not shown). We found that myeloid differentiating factor (MyD)88-deficient mice also showed enhanced CTL generation specific for OVA when coimmunized with Peptide-25 and OVA (data not shown). The MyD88-dependent signalling pathway may not be required for exerting helper activity by Peptide-25. On the whole, we do not support the hypothesis that Peptide-25 directly activates APCs.

As Peptide-25-reactive $CD4^+$ Th cells recognize MHC class II binding cognate Peptide-25 on APCs and their interaction may result in the activation of APCs, consequent to these mutual activations, APCs prime and activate $CD8^+$ CTLs specific for OVA peptides. The requirement for coimmunization of Peptide-25 and OVA in the same site suggests that the physical proximity of OVA and Peptide-25 is crucial for intracellular events in APCs. Cell-to-cell contact between Peptide-25-specific $CD4^+$ Th1 cells and APCs may be required or the two antigens need to be processed and presented by the same APC to augment the OVA-specific $CD8^+$ CTL response. To simplify the experimental system for further evaluation, we established an *in vitro* culture system using T cells from P25 TCR-Tg mice, CFSE-labelled T cells from OT-1 mice and bone-marrow-derived immature DCs propagated *in vitro*. As we reported, $CD4^+$ T cells from P25 TCR-Tg mice preferentially develop IFN-γ-producing T cells upon Peptide-25 stimulation in the presence of I-A^b splenic APCs under neutral conditions.[24] Surface expression of the MHC class I molecule, ICAM-1, CD40, CD80 and CD86 on DCs as well as IL-12p40 production was enhanced when DCs were cultured with $CD4^+$ T cells from P25 TCR-Tg mice and Peptide-25 (Fig. 8a). Furthermore, enhanced cell divisions of CFSE-labelled OT-1 T cells were observed when the cells were stimulated with OVA in the presence of DCs, Peptide-25 and $CD4^+$ T cells from P25 TCR-Tg mice (Fig. 8b). These results support the notion that $CD4^+$ Th1 cell-derived factor may augment antigen processing by APCs leading to the enhancement of the cross-priming of the antigenic peptide for $CD8^+$ T cells.

A number of approaches to augment $CD4^+$ T-cell help have been investigated.[2,10,13,39] One involves modifying the immunizing antigen itself by, for instance, haptenizing the antigen[40] or linking heterologous immunogenic peptides directly to the antigen.[17,41] The second involves coimmunization with tumour antigens and molecules with strong helper determinants.[42,43] The third, the discovery of a range of molecular signals, such as the CD40 ligand and other costimulatory signals[31–33] involved in the helper function of $CD4^+$ T cells, provides other ways to augment the $CD8^+$ T-cell response. Finally, broadly expressed wild-type molecules in murine tumour cells eliciting humoral immunity contribute to the generation of $CD8^+$ T cells and protective antitumour immune responses to unrelated tumour-specific antigen.[13]

It is important to ask whether the enhancing effect of Peptide-25 on $CD8^+$ CTL responses is beneficial, detrimental, or insignificant to the tumour-bearing host. It is likely that the phenomena that we have described in the mouse of a heightened $CD8^+$ CTL response to tumour antigens by corecognition of Peptide-25 has its counterpart in humans, occurring as a consequence of the simultaneous uptake of complex antigenic mixtures from disintegrating tumour cells by APCs. As coimmunization with Peptide-25 and tumour antigens also results in heightened resistance to tumour challenge in the mouse, this approach may be an attractive strategy for human cancer immunotherapy. In fact, there are ongoing clinical trials utilizing either heterologous helper antigens, such as keyhole limpet haemocyanin or tumour-derived helper antigens to augment antitumour immune responses by $CD8^+$ CTLs directed against tumour-derived CTL

epitopes.[44,45] As Ag85B and Peptide-25 are stimulatory to human $CD4^+$ T cells from PPD^+ healthy donors in a certain proportion of the Japanese population, Ag85B or peptide-25 may be applicable to augment antitumour immune responses by $CD8^+$ CTLs against tumour cells. Along with the scenario, we re-examined the effect of pre-immunization with *M. tuberculosis* or Peptide-25 on the antitumour CTL response and found that preimmunization of mice with heat-killed *M. tuberculosis* or Peptide-25 followed by immunization with a mixture of OVA and Peptide-25 produced a significant increase in the number of $CD8^+$ CTL s (data not shown).

While it is obvious that $CD8^+$ T cells recognize MHC class I binding peptides derived from tumour target cells based on the subsequent tumour-specific destruction, peptide derivation from tumour cells may not be an absolute requirement for $CD4^+$ T cells because they do not directly interact with tumour cells that mostly lack MHC class II expression. Co-immunization of mice with TRP-2 of B16 melanoma and Peptide-25 in IFA enhances CTL generation specific for TRP-2 (Fig. 2). However, mice that had been immunized with a mixture of TRP-2 and Peptide-25 in IFA failed to eradicate B16 melanoma, a less immunogenic tumour, although B16 melanoma growth was reduced to a certain extent (data not shown), suggesting that enhancing the effect of Peptide-25 in the TRP-2-specific CTL may not be potent enough to eradicate a less immunogenic TRP-2-expressing tumour. We need another regimen together with Peptide-25 immunization that activates effector cells leading to the eradication of tumour cells with weak immunogenicity. This notion has become extremely important in the design of future vaccines aimed at the efficient activation of both T-cell populations involved in antitumour immune responses.

Acknowledgements

We thank Drs H. Udono (Nagasaki University School of Medicine, Nagasaki, Japan), Y. Iwakura (Institute of Medical Science, University of Tokyo, Tokyo Japan), and H. Tahara (Institute of Medical Science, University of Tokyo, Tokyo Japan) for providing E.G7 cells, IFN-$\gamma^{-/-}$ mice and B16 melanoma cells, respectively, and Drs S. Takaki (Institute of Medical Science, University of Tokyo, Tokyo Japan) and S. Taki (Shinshu University, School of Medicine, Matsumoto, Japan) for their valuable suggestions throughout this study. We are also indebted to our colleagues for their critical reading of the manuscript. This work was supported by Special Coordination Funds on 'Molecular Analysis of the Immune System and Its Manipulation on Development, Activation and Regulation' for Promoting Science and Technology (K.T.), by a grant for Advanced Research on Cancer and a grant for Scientific Research on Priority Areas from the Japanese Ministry of Education, Science, Sports and Culture, and by a grant from the Uehara Memorial Foundation.

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感染症の現状と制圧戦略

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はじめに

世界の年間総死亡（2004年）は5,700万人，内訳として，循環器疾患：1,670万人，感染症：1,490万人，悪性新生物：710万人であり，感染症は総死亡の１／４強を占め，人類に大きな健康被害を招来している[1)2)]（表－１）。世界保健機関（World Health Organization，WHO）はヒト免疫不全ウイルス（HIV）感染症／後天性免疫不全症候群（AIDS），結核およびマラリアに対し，緊急事態を宣言している。加えて，非感染性疾患として

表－１　世界における死因別死亡数（2004）

死　因	死　亡　数：万人	死亡総数に対する割合：%
全　死　亡	5、700（108.4）	100（100）
循環器疾患	1、670（ 30.6）	29（ 28.0）
感　染　症	1、490（ 13.0）	26（ 12.0）
悪性新生物	710（ 32.6）	12（ 30.1）
不慮の事故死	520（ 4.0）	9（ 3.7）

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（ ）内は厚生労働省．平成17年人口動態統計月報年計（概数）の概況．

1) World Health Organization. The world health report 2005, http : //www.who.int/whr/2005/en/.

2) 厚生労働省．平成17年人口動態統計月報年計（概数）の概況．http : //www.mhlw.go.jp/toukei/saikin/hw/jinkou/geppo/nengai 05/toukei 6.html

認識されていた悪性新生物（胃癌：ヘリコバクターピロリ菌，肝細胞癌：ＢおよびＣ肝炎ウイルス，子宮頚癌：ヒト乳頭腫ウイルスなど）にも病原体感染が関与し，すなわち，感染症は広範で甚大な健康被害を惹起している。また，感染症による新たな脅威として，2001年10月，炭疽菌による生物テロリズムが現実となり，米国で肺炭疽患者の５名が死亡した[3]。

最近まで，日本を含めた先進諸国では感染症を解決された過去の疾患と錯覚し，その対策を怠ってきた。しかし，都市化による過密，人口の集中，貧困，交通機関の発達による人民の高速移動，国際化，環境破壊や温暖化など，現代社会の直面している状況が感染症の増加に関与している。感染症は病原体と宿主の生存戦争である。再興病原体は抗微生物薬に耐性を獲得し，また，新興病原体は人類に新たな脅威を提供している[4]。また，宿主側の要因として，感染抵抗力の減弱（人口の高齢化，免疫抑制薬／臓器移植や免疫疾患）が易感染性を惹起している。

これら感染症の脅威に対し，世界保健機関やGroup of Eight（Ｇ８）サミットは３大感染症，すなわち，ヒト免疫不全ウイルス感染症／後天性免疫不全症候群，結核およびマラリアを指定し，積極的に対応することを宣言している[5][6]。本稿では，感染症の脅威や制圧対策について，概説する。

１　感染症による健康被害

世界の年間総死亡（2004年）は5,700万人，内訳として，循環器疾患：

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3) Centers for Disease Control and Prevention. Public health emergency preparedness and response, http : //www.bt.cdc.gov/.

4) Morens, D. M., G. K. Folkers, and A.S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature 430 : 242-249.

5) World Health Organization. http : //www.who.int/.

6) The Okinawa summit 2000 archives, http : //www.summit-okinawa.gr.jp/e/index.htm.

1,670万人，感染症：1,490万人（総死亡の約１／４強）であることは前述したが，感染症死亡の内訳では，呼吸器感染症：396万人，後天性免疫不全症候群（結核合併を含む）：277万人，下痢性疾患：180万人，結核：156万人，マラリア：127万人である[1]（表－２）。呼吸器感染症や下痢性疾患の原因病原体は多種多様であることから，世界保健機関やＧ８サミット[5)6)]は１）ヒト免疫不全ウイルス感染症／後天性免疫不全症候群，２）結核および３）マラリア（熱帯熱マラリア）など単一病原体感染症による死亡が年間約500万人，患者発生が３億人であるため，これら３大疾患を最も重要な感染症に認定し，世界が協調して対策を構築することを宣言している（表－２）。

２　感染症の将来発生動向予測

感染症の発生動向予測として，社会基盤の整備，抗微生物化学療法やワクチンで治療・予防可能な疾患は将来的に減少することが考えられるが，その他の感染症（ヒト免疫不全ウイルス感染症／後天性免疫不全症候群や結核）は今後も現状維持あるいは増加することが予測されている。人口増

表－２　世界における感染症による死亡数（2004）

感染症	死亡数：万人
全感染症	１、４９０
急性呼吸器感染症	３９６
ＡＩＤＳ（結核合併を含む）	２７７
下痢性疾患	１８０
結核	１５６
マラリア	１２７
ワクチン予防可能疾患	１１２
性感染症	１８
髄膜炎	１７
ウイルス性肝炎（ＢおよびＣ型）	１６
熱帯性原虫・寄生虫疾患	１３
その他の感染症	１７６

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加と都市化に伴う自然環境破壊，交通機関の発達に伴う国際交流の激増は，世界の疾病構造に変化を惹起し，特定地域に発生した感染症に対し，自国の問題として迅速に対処しなければならない状況にある。このように，感染症の国際化（globalization）が進む新時代に対応するためには，民族や国境を越えた感染症対策の構築，すなわち，新時代に即応した国際協力体制の確立が必要となる[7]。

3 新興・再興感染症

新興感染症（emerging infectious diseases）は「最近約30年間（1970年以降）に，新たに発見された感染病原体，あるいは，かつては不明であった病原体により惹起され，地域的あるいは国際的に公衆衛生上問題となっている新感染症」，他方，再興感染症（re-emerging infectious diseases）は「既知感染症で，発生数が減少し，公衆衛生上ほとんど問題にならなくなっていたが，近年再び出現／増加している感染症」と定義されている[4]。

新興感染症として，30以上の感染症／病原体が報告され，代表的疾患として，クリプトスポリジウム症，エボラ出血熱，ラッサ熱，レジオネラ症，腸管出血性大腸菌感染症，ヒト免疫不全ウイルス（HIV）感染症／後天性免疫不全症候群（AIDS），H. pylori 感染症（胃炎，胃・十二指腸潰瘍，胃癌），C型ウイルス性肝炎，プリオン病（ウシ海綿状脳症，Creutzfeldt-Jakob 病），鳥型インフルエンザやウエストナイル熱（ウエストナイル脳炎を含む）や重症呼吸器症候群などがある（表－3）。再興感染症として，結核，ペスト，ジフテリア，劇症型A群溶連菌，マラリア，デング熱

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7) Center for Population and Development Studies at the Harvard School of Public Health. Burden of disease unit, http : //www.hsph.harvard.edu/organizations/bdu/index.html.

表－3 代表的な新興感染症

年	感染症
1973	ロタウイルス感染症（乳児下痢症）
1976	クリプトスポリジウム症（急性および慢性下痢症）
1977	エボラ出血熱 レジオネラ症（肺炎）
1982	腸管出血性大腸菌感染症（食中毒、溶血性尿毒症性症候群） ボレリア感染症（ライム病）
1983	*H. pylori* 感染症（胃炎、胃・十二指腸潰瘍、胃癌） ヒト免疫不全ウイルス感染症（後天性免疫不全症候群）
1989	C型ウイルス性肝炎（慢性肝炎、肝硬変、肝細胞癌）
1992	新型コレラ
1996	プリオン病（伝達性海綿状脳症、Creutzfeldt-Jakob 病）
1997	高病原性鳥インフルエンザ（H5N1）
1999	ウエストナイルウイルス感染症（脳炎を含む） ニパウイルス感染症（脳炎を含む）
2003	重症急性呼吸器症候群（SARS）

などがある[8]。

日本において，結核は最大の感染症であり，2004年の新規登録患者数：約29,700（罹患率：23.3／対人口10万人），死亡：約2,300人（死亡率：1.8／対人口10万人）であった[9]。世界的に日本は結核対策において中進国である（アメリカ合衆国：5.1，スウェーデン：4.3／対人口10万人）。結核対策上，我が国が対応を迫られている問題点として，1）一般国民，医療関係者，行政の結核に対する認識の低下，2）特異的，迅速かつ簡便な結核菌感染の検査法の開発，3）高齢結核患者の増加（70歳以上患者：約44%），4）蔓延状況の地域格差（大阪市：61.7，東京都特別区：34.7，長

8) World Health Organization. Communicable disease surveillance and response, http : //www.who.int/emc/.

9) 厚生労働省健康局結核感染症課．平成16年結核発生動向調査年報集計結果（概況），http : //www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou 03/04.html.

野県：10.4），5）集団や院内感染の続発及び増加，6）薬剤（特に，多剤）耐性結核の出現などがある。加えて，HIV/AIDS が着実に増加している現状[10]を考慮した場合，将来的には HIV/AIDS と結核の重複感染も重要な課題となることが想定される。今後，再興感染症として，結核の重要性を認識し，確実な治療や予防対策を推進することが肝要である。また，現行の結核発病予防ワクチンである bacille Calmette-Guérin(BCG)有効性に関し，根拠に基づく医療(Evidence-based medicine : EBM)の観点から，見直しが進められ，乳幼児結核（結核性髄膜炎など播種性結核）の有効性は認められたが，成人肺結核に対するＢＣＧの有効性は実証されていないため，ＢＣＧ再接種およびツベルクリン皮内反応（小学１年および中学１年時）は結核予防法を改正し，廃止した。また，現在，結核予防法の廃止（感染症の予防及び感染症の患者に対する医療に関する法律「感染症法」の改正に伴う統合）が国会で審議されている[11]。

4　薬剤耐性病原体感染症

現代における感染症の基本的制圧戦略は微生物を治療標的とした抗微生物化学療法であるが，欠点として，１）宿主への副作用（肝腎障害，造血障害など），２）薬剤耐性微生物の出現や３）環境汚染などがある。特に，薬剤耐性微生物感染症（メチシリン耐性黄色ブドウ球菌，バンコマイシン耐性黄色ブドウ球菌，ペニシリン耐性肺炎球菌，バンコマイシン耐性腸球菌，薬剤耐性緑膿菌，薬剤耐性淋菌，多剤耐性結核菌，クロロキン耐性マラリアや薬剤耐性ＨＩＶなど）は感染症対策上，重要な問題を提起している（表－４）。すなわち，人類は薬剤耐性微生物感染症に対し，武器

10）エイズ予防情報ネット．　http : //api-net.jfap.or.jp/mhw/mhw_Frame.htm.
11）厚生労働省健康局結核感染症課．　http : //www.mhlw.go.jp/topics/bukyoku/soumu/houritu/164.html

表－4 主要な抗微生物薬耐性病原体感染症

薬剤耐性病原体	感染症関連法の取扱
バンコマイシン耐性黄色ブドウ球菌（ＶＲＳＡ）	5類全数把握
バンコマイシン耐性腸球菌（ＶＲＥ）	5類全数把握
メチシリン耐性黄色ブドウ球菌（ＭＲＳＡ）	5類定点把握
ペニシリン耐性肺炎球菌（ＰＲＳＰ）	5類定点把握
薬剤耐性緑膿菌	5類定点把握
薬剤耐性淋菌	5類定点把握
クロロキン耐性マラリア	4類
薬剤耐性ＨＩＶ	5類全数把握
薬剤耐性結核菌	結核予防法

（有効な抗微生物薬）を所持していないため，対応に苦慮しているのが実情である[12]。「感染症の予防及び感染症の患者に対する医療に関する法律（感染症法）」では重要な薬剤耐性病原体感染症に対し，届出を規定している。薬剤耐性病原体感染症対策として，抗微生物薬の医療における適正使用（正確な病原体診断，薬剤感受性試験の実施，適切な抗微生物薬の選択，宿主状況の把握など），さらに，食品産業（畜産，養鶏や養魚）における抗微生物薬使用の制限が望まれる。

5 感染症の増加要因

感染症の増加要因は社会要因，宿主要因および病原体要因に大別される[4]。社会的要因として，都市化による過密，貧困，交通機関の発達による高速移動，国際化や感染症対策の軽視などが寄与している。宿主要因として，感染抵抗力の減弱（人口の老齢化，糖尿病，慢性腎不全，ヒト免疫不全ウイルス感染症／後天性免疫不全症候群，免疫抑制薬／臓器移植や免疫疾患など）が易感染性を招来している。また，病原体要因として，新興

12) World Health Organization. Anti-infective drug resistance, http://www.who.int/emc/amr.html.

病原体や薬剤耐性病原体の出現および病原性の変化などが感染症の増加に関与している（表－5）。

6　生物テロリズム

感染症による新たな脅威として，炭疽，痘瘡／天然痘，ペスト，野兎病，ボツリヌス症やウイルス性出血熱は生物テロリズムの可能性や懸念を指摘されていたが，2001年10月，炭疽菌による生物テロリズムが現実となり，アメリカ合衆国で23名の炭疽患者（吸入／肺炭疽：11名，皮膚炭疽：12名）が発生し，肺炭疽患者の5名が死亡した[3)]。さらに，炭疽菌テロリズムでは，発病者のみならず，芽胞曝露者約1万人にフルオロキノロンなど抗微生物薬による予防内服（60日間）が実施され，炭疽菌テロリズムは甚大な社会不安，混乱や被害を惹起した。すなわち，生物テロリズムの観点からも感染症危機管理体制[13)]の確立は急務の課題である。

表－5　感染症増加要因

社会要因	● 高速、海外旅行および移民の増加（年間海外旅行者：約1,700万人；熱帯地域へ500万人、海外からの旅行者：約500万人、外国人登録者：約150万人） ● 貧困や衛生状態の低下を伴う人口過密都市 ● 食料品の国際化（食料自給率：約40％） ● 地球環境破壊、気候の変化（温暖化） ● 衛生行政や感染症対策の不備や軽視 ● 生物テロ
宿主要因	● 易感染性宿主の増加（人口の高齢化、糖尿病、慢性腎不全、HIV感染症／AIDS、免疫抑制薬/臓器移植や免疫疾患など）
病原体要因	● 新興病原体の出現 ● 薬剤耐性病原体の出現 ● 病原性の変化

13）厚生労働省健康局．感染症健康危機管理実施要領，http：//www.mhlw.go.jp/general/seido/kousei/kenkou/kansen/index.html.

7 施設内（特に，院内）感染

院内感染症は「入院から48時間以降に発症した感染症」と定義されている。感染源は1）人的因子：患者，医療従事者，訪問者，また，2）環境因子：汚染医療器具／機器，食物，水，空気などである。医療の高度化，易感染者（高齢者を含む）の増加，日和見感染や薬剤耐性微生物の増加に伴い，院内感染は重要な課題となっている。院内感染の出現頻度では，1）尿路感染，2）外科的創部感染，3）呼吸器感染，4）菌血症や敗血症が多い。従って，病院は院内で発生した感染症に関し，その発生状況の把握(Hospital epidemiology)に努め，院内感染が疑われる場合，感染源や感染経路を解明することにより，感染の拡大を防止するための迅速な対応が急務となる。

施設内感染対策の基本は，手洗いの励行，清掃など施設内環境整備，施設内感染に正しい知識の啓発である。1996年，米国疾病管理防疫センター(Centers for Disease Control and Prevention : CDC)は病院内感染防止指針を提唱している[14]。その骨子は2要素，標準的予防措置(Standard precautions)および感染伝播予防措置(Transmission-based precautions)から構成されている（表－6）。

8 行政対応

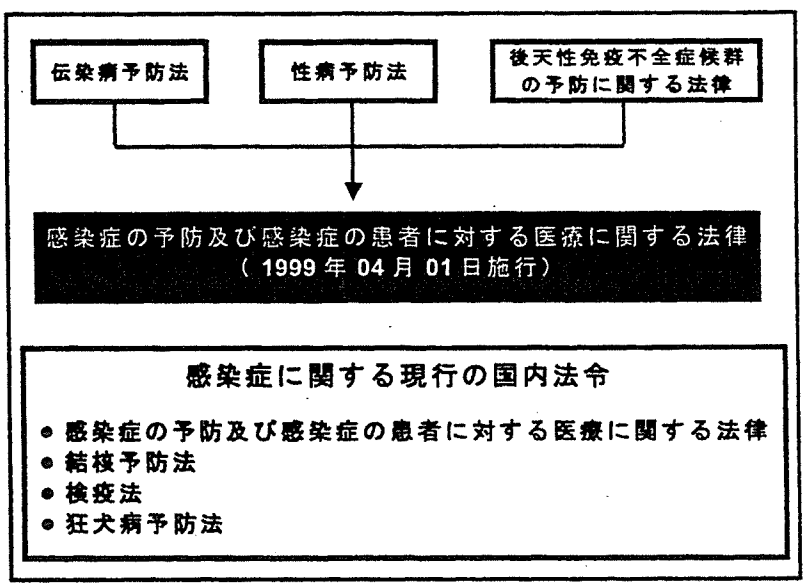
新興・再興感染症の台頭／出現，医学・医療の進歩，公衆衛生水準の向上，人権尊重と行政の透明化，事前対応型行政，国際化への対応／国際協力の推進，発生動向調査の推進および動物由来感染症対策の充実を指向

14) Centers for Disease Control and Prevention. National Nosocomial Infections Surveillance System, http : //www.cdc.gov/ncidod/dhqp/nnis_pubs.html.

表－6　院内感染防止措置

標準的予防措置（第一段階）	全ての患者（感染症および非感染症）について、医療従事者が、血液、体液、分泌物、排泄物を取り扱う際、手洗いの励行、手袋・マスク・ゴーグル・作業衣の着用を勧奨している。さらに、鋭利な医療器具に損傷・感染事故を防止するため、注射針、メス、その他、ガラス片の取り扱いに注意を喚起している。
感染伝播予防措置（第二段階）	感染症患者（接触、空気および飛沫感染）については、標準的予防措置に加えて、感染伝播予防措置を講ずる。 ● 接触感染（例：サルモネラ、赤痢、*Clostridium difficile*、黄色ブドウ球菌、疥癬など）：個室収容 ● 飛沫感染病原体感染症（例：インフルエンザ、インフルエンザ桿菌、髄膜炎菌、流行性耳下腺炎や風疹など）：個室収容やマスク着用 ● 空気／飛沫核感染病原体感染症（例：結核、麻疹、水痘、痘瘡／天然痘など）：防御的マスクの着用、換気用フィルターを装備した陰圧個室収容

し，1999年４月１日から「感染症の予防及び感染症の患者に対する医療に関する法律」(法律第114号）が施行されている。従来の「伝染病予防法」，「性病予防法」および「エイズ予防法」を廃止・統合し，わが国の感染症対策を「感染症の予防及び感染症の患者に対する医療に関する法律（感染症法)」，「結核予防法」，「狂犬病予防法」と「検疫法」に集約し，新時代に即応した感染症対策を推進している（図－１）。



図－１　日本における感染症関連法規

最近の海外における感染症の発生の状況，保健医療を取り巻く環境の変化等を踏まえ，生物テロによる感染症の発生及びまん延を防止する対策を含め，総合的な感染症予防対策を推進するため，病原体等の所持等を規制する制度を創設するとともに，入院，検疫等の措置の対象となる感染症の種類を見直すほか，入院等の措置に際しての患者への説明等の手続に関する規定を設け，あわせて結核の予防等の施策に関する規定を整備する等の必要性を鑑み，現在，「感染症法」の改正が国会で審議されている。その骨子は，１）生物テロ対策の強化，２）病原体の適正な取扱い（一～四種病原体），３）感染症患者の適切な医療と感染症予防・蔓延の防止（一～四類感染症の類型の見直し），４）結核予防法の統廃合である[11]。

おわりに

感染症は現在でも，人類に甚大な健康被害を提供し，さらに，新興・再興感染症や薬剤耐性病原体感染症は世界的に増加しており，直近においても，新型インフルエンザ出現の懸念など，油断できない状況にある[4][8]。感染症対策は感染源，感染経路および感受性宿主対策を基盤とし，１）教育・環境・行政など社会基盤整備，２）抗微生物化学療法，３）免疫介入療法，４）併用療法（抗微生物化学および免疫介入療法）が推進されている（図－２）。しかし，感染症対策の基本は予防であり，予防における最も効果的，かつ，科学的戦略は宿主を治療標的，かつ，有効活用した免疫介入療法：予防接種／ワクチンであることは過去，現在，将来共に不変であろう。事実，人類が根絶した唯一の疾患は痘瘡／天然痘であり，その勝利の原動力はワクチンであった。微生物を用いた古典的ワクチン，非細胞性成分ワクチン，遺伝子ワクチンなどの新規ワクチン，感染症に対する遺伝子治療などが登場するであろう。加えて，肝細胞癌：Ｂ型肝炎ウイルスや子宮頸癌：ヒト乳頭ウイルスではワクチン接種が実施されており，今