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Differential Requirement for CD80 and CD80/CD86-Dependent Costimulation in the Lung Immune Response to an Influenza Virus Infection[1]

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The CD28 costimulatory pathway is critical to T cell activation. Blockade of the interaction of CD28 with its ligands CD80 and CD86 using CTLA4-Ig has been proposed as a therapy for a number of immune-based disorders. We have used a murine model of influenza virus infection to study the role of CD28-dependent costimulation in the development of antiviral immune responses. In vivo treatment with CTLA4-Ig to block the interaction of CD28 with CD80 and CD86 reduced virus-specific cytotoxicity and IFN-γ production by bronchoalveolar lavage fluid CD8$^+$ T lymphocytes in vitro. It also resulted in decreased numbers of virus-specific CD8$^+$ T lymphocytes in the bronchoalveolar lavage fluid, lung, and spleen and lowered virus-specific Ab titers. Mice treated with CTLA4-Ig were able to control and clear the virus infection, but this was delayed compared with controls. Treatment with Y100F-Ig, a mutant form of CTLA4-Ig which selectively binds to CD80 and blocks the CD28-CD80 interaction leaving CD28-CD86 binding intact, did not affect Ab production, spleen cytotoxic precursors, or clearance of virus. However, Y100F-Ig treatment had a clear effect on lung effector cell function. Secretion of IFN-γ by bronchoalveolar lavage fluid CD8$^+$ T lymphocytes in vitro was decreased, and the number of virus-specific CD8$^+$ T lymphocytes in the bronchoalveolar lavage fluid and lungs of infected mice was reduced. These results indicate that CD28-dependent costimulation is important in the antiviral immune response to an influenza virus infection. The individual CD28 ligand, CD80, is important for some lung immune responses and cannot always be compensated for by CD86. *The Journal of Immunology*, **2000, 164: 79–85.**

T cells require two signals to proliferate and differentiate into effector cells. One signal is Ag specific and generated by the interaction of the TCR with antigenic peptide bound to MHC molecules on APC. The second, or costimulatory, signal is principally generated through engagement of the CD28 receptor on T cells with its ligands CD80 and CD86 on APC (reviewed in Ref. 1). It is unclear why two costimulatory ligands exist and whether they mediate distinct roles during immune responses.

CTLA4-Ig is a soluble chimeric protein consisting of the extracellular domain of CTLA-4 fused to the Fc portion of human IgG1. This molecule has been shown in a number of models to be powerfully immunosuppressive, through its ability to block CD28 interaction with CD80 and CD86. CTLA4-Ig has been shown to prevent organ transplant rejection (2, 3), lupus-like autoimmune disease (4), experimental autoimmune encephalomyelitis (5, 6), autoimmune oophoritis (7), and asthmatic lung inflammation (8) in experimental models. In addition, CTLA4-Ig has been proposed as a therapeutic agent for a number of immune-based disorders, and phase I clinical trials in patients with psoriasis vulgaris have recently been completed (9). If CTLA4-Ig is to be used in a clinical situation, its effect on clearance of infectious agents must be established. We have examined the antiviral immune response in a murine model of influenza infection during concurrent treatment with CTLA4-Ig.

Y100F-Ig is a mutant form of CTLA4-Ig that selectively binds CD80 and blocks CD28-CD80 interactions but leaves CD28-CD86 binding intact (10). We have previously shown in a model of Ag-induced airway eosinophilia that Y100F-Ig prevents the accumulation of eosinophils and lymphocytes in the lungs of immunized mice, but does not block Ag-induced systemic blood eosinophilia or IgE Ab production (10). These results suggested that CD80-dependent costimulation may be especially important for the development of immune responses in the lung. Further in vitro evidence also suggests that CD80 may be critical for the activation of T lymphocytes in the lung. The T cell stimulatory capacity of murine lung dendritic cells in vitro is reported to be almost exclusively dependent on expression of CD80 while CD86, although expressed, has a secondary role (11). To determine whether blockade of CD80-dependent costimulation can also inhibit other lung immune responses, we investigated the effect of Y100F-Ig treatment during an influenza virus infection.

Materials and Methods

Mice

C57BL/6J mice were bred and maintained at the Biomedical Research Unit of the Wellington School of Medicine. All animal experimental procedures used in this study were approved by the Wellington School of Medicine Animal Ethics Committee and conducted in accordance with the guidelines of the University of Otago (Dunedin, New Zealand).

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Reagents

Chimeric proteins derived from the fusion of the extracellular portion of human or murine CTLA-4 to human IgG1 were used in these studies (12). Y100F-Ig is a mutant CTLA4-Ig in which the tyrosine at position 100 is substituted with phenylalanine (10). CTLA4-Ig and Y100F-Ig bind CD80 with indistinguishable kinetics, whereas binding of Y100F-Ig to CD86 is undetectable (10). The mAb L6-Ig, which has a human Fc region, was used as a control (12). Mice were injected i.p. with 200 μg of murine CTLA4-Ig, Y100F-Ig, or L6-Ig or 400 μg of human CTLA4-Ig, Y100F-Ig, or L6-Ig, beginning the day before infection, and continuing every 48 h throughout the experiment.

The H-2D^b-binding influenza A peptide NP366-374 (ASNENMETM; see Ref. 13) was synthesized by Chiron Technologies (Victoria, Australia).

Virus

The A/HKx31 (H3N2) influenza A virus is a laboratory-generated recombinant with the external surface components of A/Aichi/2/68 (H3N2) and the internal components of A/PR8/8/34 (H1N1) (14). Virus stocks were grown in the allantoic cavities of 10-day-old embryonated chicken eggs and stored as infectious allantoic fluid at −80°C. Virus stock was shown to be endotoxin free by the *Limulus* amebocyte lysate assay (Sigma, St. Louis, MO). Virus titer was determined by agglutination of human erythrocytes and is expressed as hemagglutinating units (HAU).[3] Mice were anesthetized by an i.p. injection of a mixture of ketamine and xylazine (Phoenix, Auckland, New Zealand), and 12 HAU of virus in a 30-μl volume of PBS was administered by intranasal inoculation.

Tissue sampling

Mice were anesthetized and exsanguinated from the vena cava. The trachea was cannulated and a bronchoalveolar lavage (BAL) performed with three consecutive washings with 0.8 ml of PBS, each of which was infused and withdrawn five times. BAL cells from individual mice were pooled, erythrocytes were lysed in 0.14 M NH_4Cl and 17 mM Tris-HCl, and nucleated cells were adhered on plastic for 2 h at 37°C to remove macrophages. Lungs were perfused via the heart right ventricle with 10 ml of PBS, and minced lung tissue was digested with 2.4 mg/ml collagenase (Life Technologies, Auckland, New Zealand) and 0.1% DNase I (Sigma) for 1 h at 37°C. Lung-infiltrating lymphocytes were purified over a 65% Percoll gradient and the interphase cells were collected. Single-cell suspensions were prepared from spleen by teasing though nylon gauze, and erythrocytes were lysed.

Virus titers in lung tissue

Lungs were removed at various intervals after infection and stored at −80°C until further assay. Individual lung samples were homogenized in 1 ml of IMDM (Life Technologies). Homogenates were diluted serially 10-fold in IMDM supplemented with 2 μg/ml trypsin and plated in triplicate onto Madin-Darby canine kidney cells (15). The cultures were incubated for 7 days at 35°C. The culture supernatants were tested for the presence of viral hemagglutinin activity by mixing 50 μl of supernatant with 50 μl of a 0.4% suspension of human erythrocytes. Virus titers were determined by interpolation of the last dilutions that showed hemagglutination.

Cytotoxicity assay

The cytotoxic activity of BAL and spleen cells was determined using the just another method (JAM) test (16) on 5000 labeled EL4 cells that had been incubated in the presence or absence of 10 μg/ml $NP_{366-374}$ peptide for 1 h at 37°C before the assay as described (17). The percentage of cytotoxicity was calculated from the mean of triplicate wells. BAL cells were tested directly ex vivo for cytotoxic activity. Spleen cells were first stimulated in vitro with 0.1 μM $NP_{366-374}$ peptide for 5 days. All cultures were in IMDM containing 2 mM glutamine, 1% penicillin-streptomycin, 5×10^{-5} M 2-ME (Sigma), and 5% FCS (Life Technologies).

FACS Analysis

Anti-FcγRII (2.4G2), anti-CD8 (2.43), and anti-CD4 (GK1.5) Ab were affinity purified from tissue culture supernatants using protein G-Sepharose (Pharmacia Biotech, Uppsala, Sweden) and conjugated with biotin. Anti-Vβ8.3-FITC was obtained from PharMingen (San Diego, CA). Cells were stained in PBS containing 2% FCS and 0.01% sodium azide as described (10) and analyzed on a FACSort (Becton Dickinson, Mountain View, CA).

[3] Abbreviations used in this paper: HAU, hemagglutinating units; BAL, bronchoalveolar lavage; JAM, just another method.

Enzyme-Linked Immunospot

Polyvinyl chloride 96-well plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 20 μg/ml AN18 (anti-IFN-γ) and blocked with 10% BSA in PBS for 60 min at room temperature. After washing, freshly explanted cells were added in serial dilutions. Cells were incubated with or without 10 μg/ml of the peptide $NP_{366-374}$ for 6 h at 37°C in a 5% CO_2 incubator. After removal of cells, an appropriate dilution of the secondary Ab XMG-D6-biotin was added and incubated at 4°C overnight. Alkaline phosphatase-avidin conjugate was added and incubated for 1 h at 37°C; spots representing single IFN-γ-producing cells were revealed using the substrate 5-bromo-4-chloro-3-indolyl phosphate (Sigma) and counted under an inverted microscope.

Ab ELISA

Polyvinyl chloride 96-well plates (Nunc) were coated overnight at 4°C with 10 μg/ml purified Influenza x31 Ag (SPAFAS, Storrs, CT), and ELISA reactions were conducted as described previously (8). Ab titers are expressed in U/ml (reciprocal of 50% Ab titer).

Results

Effect of CTLA4-Ig and Y100F-Ig treatment on CD8$^+$ T cell effector function

To determine the importance of CD28-dependent costimulation during an influenza virus infection, mice were treated with i.p. injections of CTLA4-Ig, or the isotype control L6-Ig, every 48 h beginning the day before infection. To assess the role of the individual CD28 ligand CD80, mice were treated with a mutant form of CTLA4-Ig, Y100F-Ig, which does not bind to CD86 (10). Neither Y100F-Ig nor CTLA4-Ig affected the total number of CD8$^+$ T lymphocytes detected in the BAL of mice infected with influenza virus at day 9 after infection. In contrast, treatment with CTLA4-Ig, but not Y100F-Ig, considerably reduced the number of CD4$^+$ T lymphocytes in the BAL as compared with the L6-Ig treated group (data not shown). A JAM test using EL4 cells coated with the immunodominant peptide $NP_{366-374}$ was used to detect virus-specific CTL. BAL cells from mice treated with CTLA4-Ig exhibited no cytotoxic activity (Fig. 1). No cytotoxic activity was detected from BAL cells taken at day 12 after infection (data not shown), indicating that lack of cytotoxicity was not due to a delay in the kinetics of the appearance of CTLs in mice treated with CTLA4-Ig. The effect of Y100F-Ig on the cytotoxic activity of BAL lymphocytes was variable. In some experiments, the cytotoxic activity, although slightly diminished, was comparable to that of controls (Fig. 1), whereas in other experiments, it was partially reduced (data not shown).

To distinguish between an effect of CTLA4-Ig on CD8$^+$ T cell priming, as opposed to an effect on the acquisition of cytotoxic effector function in vivo, spleen cells from infected mice were restimulated in vitro in the presence of specific peptide to allow the differentiation of CTL precursors into effector cells before assay in a JAM test. Cultures of spleen cells from mice treated with Y100F-Ig showed equivalent killing to cultures from L6-Ig controls (Fig. 2). In contrast, very little killing activity was detected from spleen cell cultures from mice treated with CTLA4-Ig (Fig. 2). This suggests that CTLA4-Ig causes a severe defect in the priming and/or expansion of virus specific CD8$^+$ T cells during influenza virus infection.

As an independent assay of CD8$^+$ T lymphocyte function, enzyme-linked immunospot assays were used to evaluate the number of Ag-specific IFN-γ-secreting cells. BAL lymphocytes were collected at day 9 after infection, restimulated in culture in the presence or absence of $NP_{366-374}$ peptide for 6 h, and the number of IFN-γ-secreting cells counted. High numbers of IFN-γ-producing cells could be demonstrated in the BAL of control L6-Ig-treated mice, but only in the presence of $NP_{366-374}$ peptide, indicating that cytokine production was Ag specific (Fig. 3). Very few

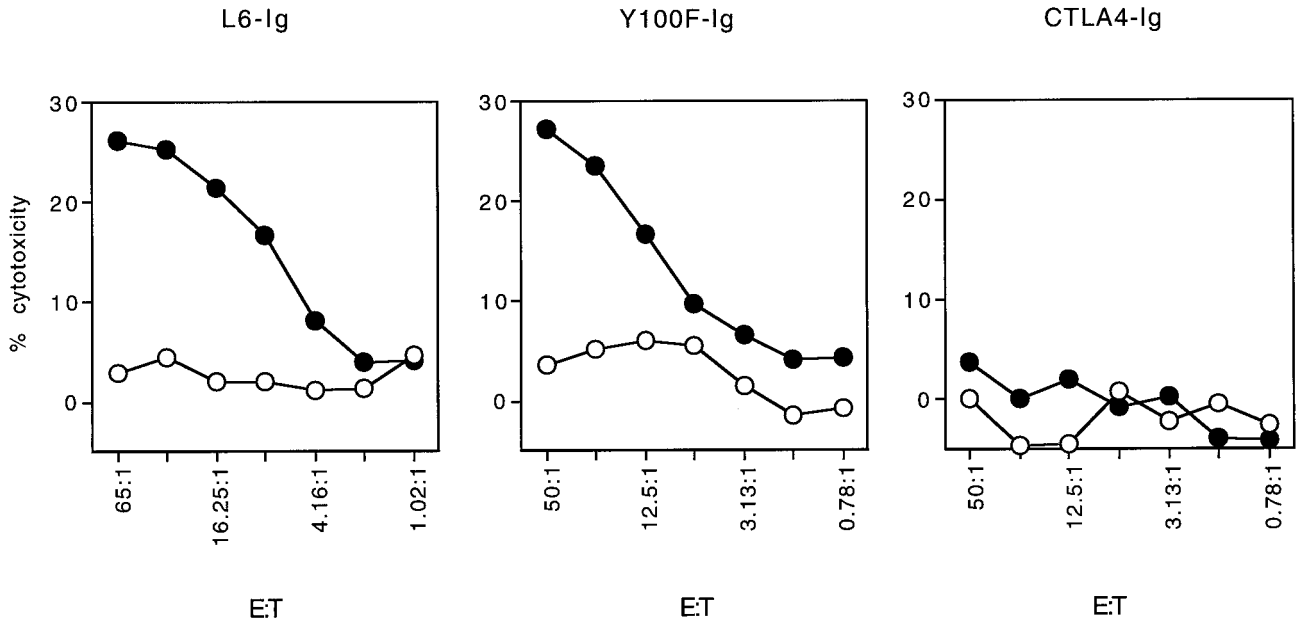

FIGURE 1. Treatment with CTLA4-Ig abrogates the cytotoxic activity of BAL lymphocytes, whereas Y100F-Ig has marginal effects. C57BL/6J mice (five per group) were infected intranasally with 12 HAU of influenza virus. Mice were treated i.p with either CTLA4-Ig, Y100F-Ig, or L6-Ig every 48 h beginning the day before infection. BAL cells were collected on day 9 after infection, pooled, and depleted of macrophages by plastic adherence. Cytotoxic activity was measured using a JAM test. The filled symbols represent killing of EL4 target cells coated with $NP_{366-374}$, and the open symbols represent killing of EL4 targets without peptide.

lymphocytes were found in the BAL of noninfected mice, and no IFN-γ-secreting cells were detected in lung-infiltrating lymphocytes isolated from noninfected mice (data not shown). The number of cytokine-secreting cells was severely reduced in mice treated with CTLA4-Ig although some effector function was detected (Fig. 3). This is in contrast to the cytotoxic assay where no CTL activity was apparent after CTLA4-Ig treatment. In two separate experiments, Y100F-Ig reduced the number of IFN-γ-secreting cells in the BAL compared with controls (Fig. 3), indicating that CD80-dependent costimulation was required for the optimal development of IFN-γ-secreting cells. The numbers of IFN-γ-secreting $CD8^+$ T cells specific for $NP_{366-374}$ in lymph node and spleen were too low to allow a meaningful comparison.

Effect of CTLA4-Ig and Y100F-Ig on the proportion of Vβ8.3 TCR^+ lung lymphocytes

Because only a small percentage of lymphocytes in the BAL are Ag specific (18), it is possible that CTLA4-Ig and Y100F-Ig are

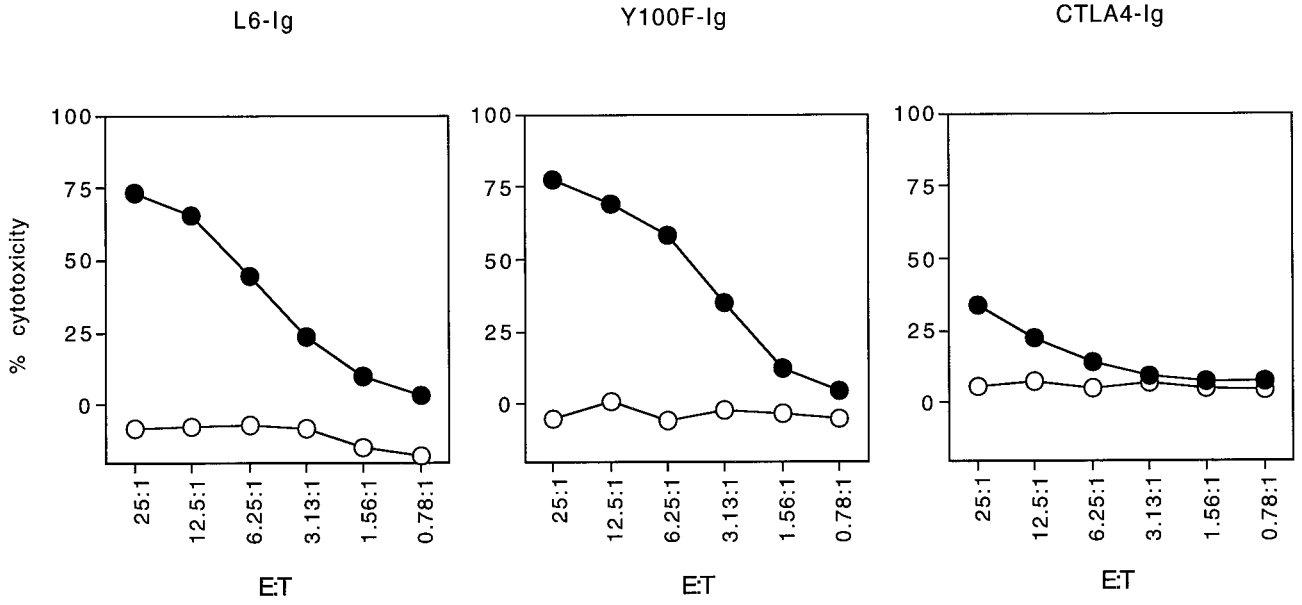

FIGURE 2. Treatment with CTLA4-Ig markedly reduces the cytotoxicity of in vitro stimulated splenocytes, whereas Y100F-Ig has no effect. Mice (four per group) were treated with CTLA4-Ig, Y100F-Ig, or L6-Ig and infected with influenza virus as detailed in the legend to Fig. 1. Splenocytes were obtained from groups of mice 9 days after infection and restimulated in vitro with 0.1 μM $NP_{366-374}$. After 5 days of culture, cells were tested for cytotoxic activity by a JAM test. The filled symbols represent killing of EL4 target cells coated with $NP_{366-374}$, and the open symbols represent killing of EL4 targets without peptide.

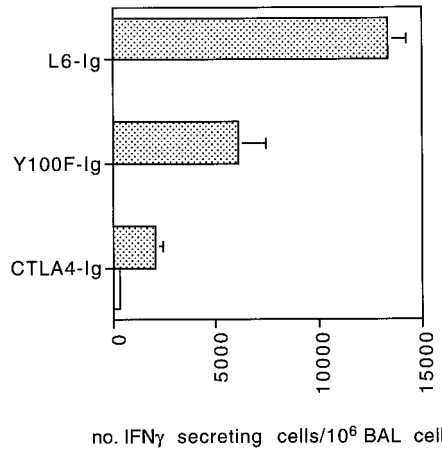

FIGURE 3. Treatment with CTLA4-Ig or Y100F-Ig reduces the number of Ag-specific IFN-γ-secreting cells in the BAL. Mice (three per group) were treated with CTLA4-Ig, Y100F-Ig, or L6-Ig and infected with influenza virus as detailed in the legend to Fig. 1. Freshly isolated BAL cells were collected on day 9 after infection, pooled, and depleted of macrophages by plastic adherence. The filled bars represent the number of IFN-γ-secreting cells/10^6 cells after a 6-h restimulation with $NP_{366-374}$. The open bars represent the number of IFN-γ-secreting cells/10^6 cells after a 6-h incubation without peptide.

inhibiting either the infiltration of Ag-specific cells into the lung or the further activation of cells to effector function once they are in the lung. Therefore, we sought to determine the proportion of Ag-specific cells in the BAL. Cells taken directly from the BAL of infected mice exhibit a preferential accumulation of $V\beta8.3^+CD8^+$ T cells (19), as the Vβ8.3 TCR is selectively overrepresented in the $CD8^+$, $NP_{366-374}$-specific population. As an estimate of the proportion of Ag-specific cells, we measured the expression of Vβ8.3 on BAL lymphocytes by FACS analysis. As expected, the usage of the Vβ8.3 TCR on $CD4^+$ cells was equivalent in all treatment groups and did not change over the time course of the infection (Fig. 4). In contrast, the proportion of Vβ8.3-expressing cells in the $CD8^+$ population was significantly increased in the L6-Ig-treated group compared with noninfected mice. The proportion of Vβ8.3 cells was increased at day 9 and further expanded at day 13 (Fig. 4), presumably as a result of more rapid clearance of non-virus-specific cells from the lung. Infected mice treated with CTLA4-Ig had marginal or no increase in the proportion of $V\beta8.3^+CD8^+$ cells in both the lung and BAL. This suggests that very few $NP_{366-374}$-specific $CD8^+$ T cells are present in the lung and BAL fluid of CTLA4-Ig-treated mice. Finally, an increase in the proportion of $V\beta8.3^+CD8^+$ cells was detected in both the lung and BAL fluid of Y100F-Ig-treated mice (Fig. 4). This increase was usually, but not always, less profound than the increase observed in control L6-Ig-treated mice (data not shown), suggesting that lower numbers of Ag-specific cells were present in the lung and BAL fluid of Y100F-Ig-treated mice.

Effect of CTLA4-Ig and Y100F-Ig on Ab production

To determine the requirement of CD28-dependent costimulation for Ab production, virus-specific IgG1 and IgG2a titers were measured in CTLA4-Ig, Y100F-Ig, and L6-Ig-treated mice using an

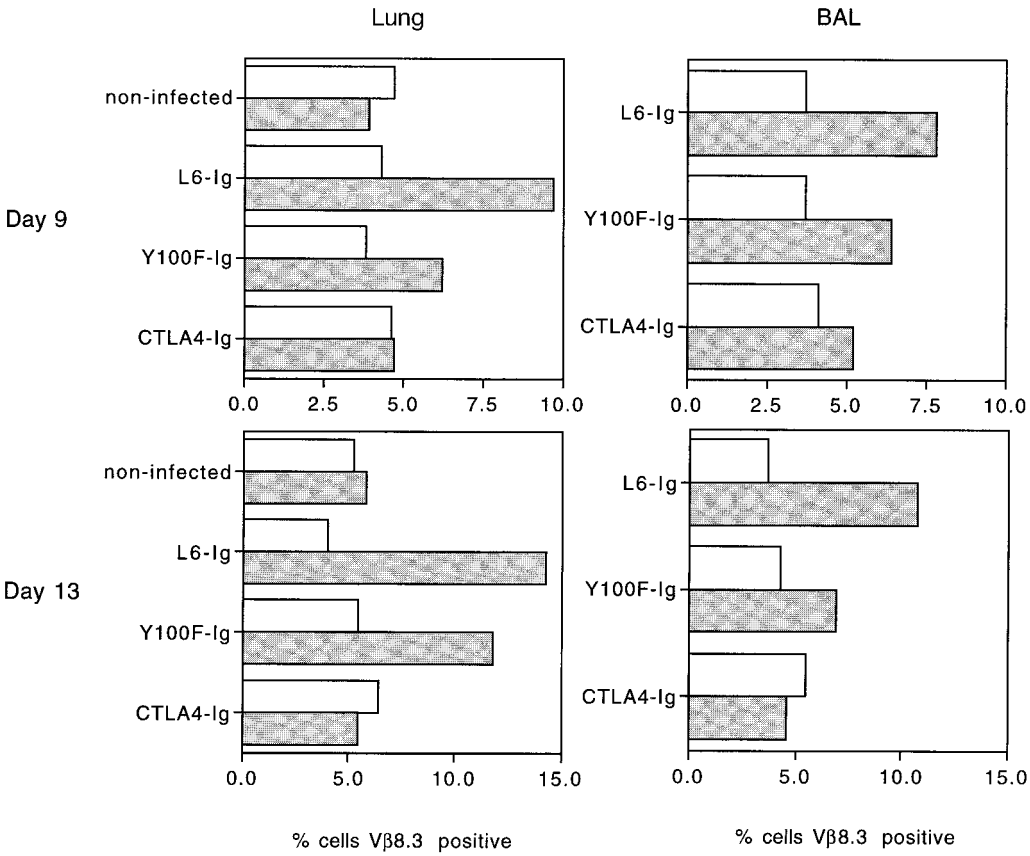

FIGURE 4. Treatment with CTLA4-Ig or Y100F-Ig reduces the accumulation of $V\beta8.3^+CD8^+$ cells in the BAL and lung. Mice (three per group) were treated with CTLA4-Ig, Y100F-Ig, or L6-Ig and infected with influenza virus as detailed in the legend to Fig. 1. Freshly isolated BAL cells and lung-infiltrating lymphocytes were collected at the indicated time points after infection, pooled, and depleted of macrophages by plastic adherence. The percentages of $CD4^+$ cells (open bars) and $CD8^+$ cells (filled bars) which were $V\beta8.3^+$ were determined by FACS analysis.

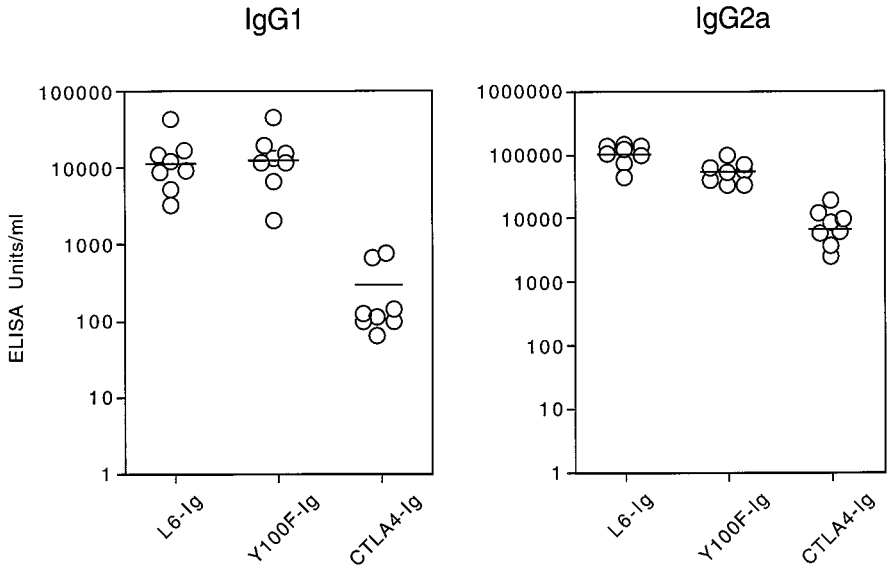

FIGURE 5. Treatment with CTLA4-Ig reduces the titer of virus-specific IgG1 and IgG2a, whereas Y100F-Ig has no effect. Mice were treated with CTLA4-Ig, Y100F-Ig, or L6-Ig and infected with influenza virus as detailed in the legend to Fig. 1. Serum was collected at day 12 after infection, and virus-specific Ab levels were determined by ELISA. Symbols represent serum Ab titers from individual mice; the horizontal line represents the geometric mean for each group.

ELISA. Results showed that both IgG1 and IgG2a were lowered by CTLA4-Ig treatment (Fig. 5). The mean titer of IgG1 was 30-fold lower than in the L6-Ig controls, and the mean titer of IgG2a was 7-fold lower. Levels of IgG1 and IgG2a were similar in Y100F-Ig- and L6-Ig-treated mice (Fig. 5). Serum from noninfected mice had undetectable levels of both IgG1 and IgG2a (data not shown). Mice depleted of $CD4^+$ T cells (20) and mice deficient in expression of MHC class II molecules (J. M. Lumsden and F. Ronchese, unpublished data) are still able to produce influenza-specific Abs, especially IgG2a, albeit at a reduced level. This suggests that a component of the Ab response to influenza virus infection is $CD4^+$ T cell independent, which may explain why reasonable titers of Ab were detected even when CD28-dependent costimulation was blocked.

Effect of CTLA4-Ig and Y100F-Ig treatment on virus clearance

Influenza virus clearance is mediated by $CD8^+$ cytotoxic T cells, but other mechanisms such as Ab can clear the infection when $CD8^+$ cells are absent (21, 22). To determine whether the defects in T cell activity observed in mice treated with CTLA4-Ig or Y100F-Ig influenced virus clearance, infectious virus titers were measured at days 6 and 9 after infection. As shown in Fig. 6, Y100F-Ig did not alter the rate of clearance of virus from infected lungs. In contrast, mice treated with CTLA4-Ig showed delayed virus clearance. In other experiments, we found that all mice treated with CTLA4-Ig had cleared the virus by day 12, indicating that virus clearance is ultimately achieved in CTLA4-Ig-treated mice (data not shown).

Discussion

The aim of the present study was to establish whether blockade of CD80/CD86-dependent costimulation affects the development of an anti-influenza immune response, and more specifically, to establish the relative importance of the CD80 costimulatory molecule in the lung immune response. Our experiments revealed that both CTLA4-Ig and Y100F-Ig had significant effects on the anti-influenza virus immune response.

CTLA4-Ig-treated mice had decreased Ab production, no detectable ex vivo cytotoxic activity, and reduced Ag-specific secretion of IFN-γ by BAL lymphocytes. The defect in $CD8^+$ T cell function was likely to be due to lack of priming and/or expansion of virus specific $CD8^+$ T cells, since the proportion of $CD8^+$ T cells expressing a Vβ8.3 TCR, which is preferentially expressed on $NP_{366-374}$-specific $CD8^+$ T cells, was not increased. In addition, no cytotoxic activity was detected even after lymphocyte culture in vitro in the presence of specific peptide, to allow the activation of CTLp to effector cells. We think these effects are due to a direct effect of CTLA4-Ig on $CD8^+$ T cell activation, rather than to an indirect effect through inhibition of $CD4^+$ T cell help. Mice that are deficient in $CD4^+$ T cells have an equivalent level of CTL activity in the BAL compared with wild-type mice and are able to eliminate influenza virus from the lung with near to normal kinetics (Refs. 23 and 24 and J. M. Lumsden and F. Ronchese, unpublished results).

Immune responses to viral infectious agents are variously affected by the blockade of the CD28 costimulatory pathway. $CD28^{-/-}$ mice (25) and mice transgenic for a soluble form of CTLA4-Ig (26) infected with lymphocytic choriomeningitis virus generated a normal CTL response. In contrast, infection with vesicular stomatitis virus did not induce any measurable CTL activity. It was suggested that the requirement for CD28 costimulation correlates inversely with viral replication and that high Ag

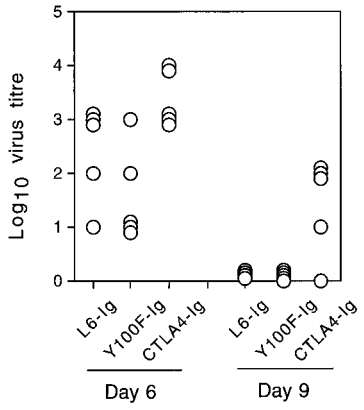

FIGURE 6. Treatment with CTLA4-Ig delays the clearance of virus from infected lungs, whereas Y100F-Ig has no effect. Groups of mice were treated with CTLA4-Ig, Y100F-Ig, or L6-Ig and infected with influenza virus as detailed in the legend to Fig. 1. Titers of infectious HKx31 virus in lung extracts, obtained from individual mice at the indicated time points after infection, were determined in the Madin-Darby kidney cell assay. Symbols represent virus titers from individual mice.

expression driven by these infectious agents may decrease the dependency on the CD28 costimulatory signal, essentially by increasing the strength of the Ag-specific "signal one." Influenza virus causes a transient infection of epithelial cells in the respiratory tract, but causes no productive infection of cells in other tissues. This localized infection would be likely to produce a lower level of Ag than viruses such as lymphocytic choriomeningitis virus and may explain why CTLA4-Ig can inhibit the immune response during an influenza infection. However, some T cell activation must have occurred in mice treated with CTLA4-Ig. Mice lacking T cells cannot control an influenza virus infection (27, 28), whereas mice treated with CTLA4-Ig were able to control and clear the virus, although with delayed kinetics. The observed low level of IFN-γ production by BAL cells also supports a limited degree of T cell activation in CTLA4-Ig-treated mice.

In previous studies, we have reported that CD80 is essential to the development of allergic lung inflammation in a murine model (10). Treatment with Y100F-Ig drastically inhibited the development of Ag-dependent airway eosinophilia, but had no effect on blood eosinophilia or Ab production, indicating a preferential effect on lung immune responses. A similar role for CD80 is also suggested by the observations reported in this study. The cytotoxic activity of BAL $CD8^+$ lymphocytes was reduced, sometimes only marginally (Fig. 1), sometimes more significantly (data not shown). The number of IFN-γ-secreting cells in the BAL was also reduced, although not as severely as in CTLA4-Ig-treated mice. Finally, the proportion of $V\beta8.3^+CD8^+$ cells in the lung and BAL of infected mice treated with Y100F-Ig was generally, but not always, reduced compared with that of controls. In contrast to this partial but still clear effect on lung immune responses, Y100F-Ig had no effect on systemic antiviral immune responses, as measured by spleen cytotoxic activity and Ab titers. These data suggest that the function of CD80 in lung immune responses is not as easily compensated for by CD86, as it is in the case of systemic immune responses. The mechanism for this effect of Y100F-Ig is still unclear, but one mechanism may be to inhibit the proliferation of Ag-specific cells in the draining lymph nodes. More accurate estimation of the numbers of Ag-specific cells using tetrameric MHC class I reagents may elucidate this. It is also possible that the reduced cytotoxicity and IFN-γ production by BAL $CD8^+$ cells was caused by a defect in the acquisition of effector function once in the lung. The primary APC presenting viral Ags in the lung is likely to be the dendritic cell. Masten et al. (11) reported that the T cell stimulatory capacity of murine lung dendritic cells in vitro is almost exclusively dependent on expression of CD80, whereas CD86, although expressed, has a secondary role. This may provide another hypothesis as to the mechanism by which Y100F-Ig affects lung effector function. It would also be interesting to see whether increased expression of CD86 on lung APCs would overcome the requirement for CD80 for complete lung effector cell activity.

Treatment with CTLA4-Ig and Y100F-Ig appeared to affect differentially $CD8^+$ T cell functions such as cytotoxicity and IFN-γ production. CTL activity was completely inhibited by CTLA4-Ig treatment. In contrast, IFN-γ production was still demonstrable although at a much reduced level. The lack of cytotoxic activity by $CD8^+$ cells may reflect a lower sensitivity of this assay or may indicate a higher dependence of cytotoxic function on costimulatory signals. Other cases of differential costimulatory requirements for different T cell functions have been reported in the literature. The absence of costimulation during the in vitro activation of a $CD8^+$ T cell clone had differential effects on certain TCR-dependent effector functions (29). In that study, cytotoxic activity was not affected while IFN-γ production was reduced. Recently, transgenic $CD8^+$ T cells rendered anergic in vivo were shown to produce IFN-γ in vivo but were unable to proliferate or to kill target cells in vitro (30, 31). The difference in the activation status of the T cells examined in the above studies (T cell clones vs naive T cells) may explain the discrepancies in the observed effects. It is interesting that, in our study, Y100F-Ig had an inverse effect on $CD8^+$ T cell function as compared with CTLA4-Ig. CTLA4-Ig completely blocked cytotoxic activity but left some IFN-γ production. Y100F-Ig had a consistent effect on IFN-γ production, but affected cytotoxicity less extensively and less reproducibly. Again, this suggests that although CD86 may provide sufficient costimulation for some T cell functions, CD80 serves a specific function and is required for some T cell activities. Since CD80 is reported to be up-regulated slowly by APC (32, 33), its predominant role may be in late T cell effector functions. Indeed, a recent paper (34) described how acquisition of CTL activity by $CD8^+$ cells requires only one cell division, while IFN-γ production is a late event (35).

In conclusion, we show that CD28-dependent costimulation is critical to the development of an effective anti-influenza virus immune response. We also present evidence in favor of an important role of CD80 in lung immune responses, confirming the hypothesis that CD80 and CD86 molecules have differential roles in the development of immune responses.

Acknowledgments

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