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Central to the pathogenesis of allergic airway inflammation are the activation and differentiation of T lymphocytes. This process requires the participation of the CD28 costimulatory receptor. Blockade of CD28 has been demonstrated to prevent inflammation and airway hyperreactivity in a murine model of asthma. Whether this is due specifically to defects in initial T cell activation or whether effector responses are also impaired has not been determined. Using adoptive transfer studies of Ag-specific lymphocytes, we demonstrate that CD28 has a critical role in both the induction and effector phase of allergic airway inflammation. Transfer of in vitro activated and Th2-differentiated Ag-specific lymphocytes from wild-type hosts restored inflammation, but not tissue eosinophilia in CD28-deficient recipients. Furthermore, similarly activated and differentiated CD28-deficient lymphocytes were ineffective at mediating inflammation in wild-type recipients. Secondary cytokine and proliferative responses of activated Th2 cells were highly dependent on CD28 in vitro. Moreover, eosinophil recruitment to both the lung and peritoneum is impaired by the lack of CD28, suggesting a generalized defect in the ability of eosinophils to accumulate at sites of inflammation in vivo. These data identify a novel role for CD28 in the effector phase of allergic airway inflammation and suggest that inhibition of this pathway may be a useful therapeutic intervention in previously sensitized individuals. The Journal of Immunology, 2004, 173: 632–640.

Th cell activation requires coordinate signaling through both Ag and costimulatory receptors. The CD28 receptor is one of the most important and best characterized costimulatory molecules. Ligation of CD28 on naive T cells substantially increases cytokine production and cell proliferation. CD28 signaling also promotes T cell survival through PI3K-dependent activation of protein kinase B and induction of Bcl-xL (1). The importance of CD28 during an in vivo immune response has been characterized in several systems, including a murine model of allergic asthma (2). A strict requirement for this pathway has been demonstrated in the induction of allergic airway inflammation by administration of soluble blocking reagents and by challenge of mice deficient in CD28 (3–6).

Most studies of CD28 have focused on the initiation of immune responses. Although proximal events in T cell activation have been shown to be profoundly affected by CD28, later responses were thought to be less dependent upon CD28. Early studies of an in vitro model of immunologic tolerance demonstrated that activation of T cells in the absence of costimulation rendered them refractory to further antigenic challenge, leading to the hypothesis that CD28 was indispensable for successful T cell activation (7, 8). However, studies of CD28-deficient mice have shown that some T cell responses are preserved (9, 10). Consistent with this, the identification of other costimulatory molecules such as ICOS supported a model in which CD28 functioned during the initial events of T cell activation, while other costimulatory receptors modulated later phases of the immune response (11–13).

We specifically examined whether the CD28 dependence of allergic airway inflammation was due to a requirement for CD28 in the initial or effector phases of the immune response. We found that while CD28 played a critical role in the initial activation and clonal expansion of Ag-activated T cells, it was also required for effector function at the site of Ag challenge in the lung. Thus, therapeutic strategies targeted at this pathway may be effective in the treatment of diseases in which T cell sensitization has already occurred.

Materials and Methods

Mice

BALB/cByJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). DO11.10 TCR transgenic mice that recognize OVA peptide (OVA323–339) (14) were obtained from K. Murphy (Washington University, St. Louis, MO). CD28-deficient mice on the DO11.10 or BALB/c background were obtained originally from S. Reiner and C. Thompson (University of Pennsylvania, Philadelphia, PA). All mice were bred and housed under specific pathogen-free conditions at Washington University School of Medicine. All animal experimentation has been approved by the Institutional Animal Use and Care Committee of Washington University School of Medicine.

Antibodies

Fluorescently conjugated Abs against the DO11.10 TCR (clone KJ1–26) were purchased from Caltag Laboratories (Burlingame, CA). ELISA kits for IL-13, IL-4, and eotaxin were purchased from R&D Systems (Minneapolis, MN), and samples were analyzed according to the manufacturer’s protocol. The hybridoma expressing anti-IL-12 was obtained from E. Unanue (Washington University), and purified Ab was obtained by affinity chromatography of culture supernatant over a protein G column. All other Abs were purchased from BD Pharmingen (San Diego, CA).

Th cell differentiation

Splenocytes were isolated from CD28+/+ or CD28−/− DO11.10 mice and activated with 0.3 μM OVA323–339 peptide in the presence of rIL-4 (50 ng/ml; purchased from R&D Systems) and anti-IL-12 (5 μg/ml), as previously described (15, 16). Three days following activation, the cultures were expanded by addition of medium and exogenous cytokine. At day 7 following initial activation, the cytokine profile of the cells was verified by intracellular staining for IL-4 and IFN-γ using standard methods.

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samples were analyzed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences, San Jose, CA). In some experiments, the cells were rested overnight in fresh medium without Ag or cytokine and restimulated with OVA peptide for 48 h. Replicate plates were analyzed for proliferation by thymidine incorporation or for IL-4 secretion by ELISA.

Induction of allergic airway inflammation

Mice were immunized on days 0 and day 7 i.p. with OVA (Sigma-Aldrich, St. Louis, MO) adsorbed to alum, as previously described (17). On day 14, the mice were challenged with 50 μl of 2% OVA in PBS intranasally in the morning and afternoon. Three days following inhaled challenge, the mice were euthanized and tissues were collected for analysis. In adoptive transfer experiments, naïve BALB/c mice, either CD28−/− or CD28+/+, received 2 × 10^5 Th2-differentiated DO11.10 T cells by i.v. injection. The following day, the mice were challenged intranasally with OVA, as described above. Bronchoalveolar lavage (BAL) was performed by intratra- cheal installation of PBS + 1% BSA. Flow cytometry was performed on the recovered cells to determine the percentage of CD4+ and/or KJ1-26+ T cells. Cytospin preparations were stained with a modified Wright-Giemsa stain, and manual differentials were performed. BAL fluid was stored at −80°C and subsequently analyzed for cytokine or chemokine expression by ELISA, according to the manufacturer’s protocol. For histologic analysis, the lungs were inflated with neutral buffered formalin to 25-cm water pressure and fixed overnight. The samples were progressively dehydrated in ethanol and processed for sectioning and staining with H&E. In selected experiments, one lung was inflated with 50% OCT in PBS and frozen on dry ice. Frozen sections were examined for eosinophils by staining for the presence of cyanide-resistant peroxidase activity, followed by counterstaining with methyl green, as previously described (6). For isolation of RNA, one lung was frozen on dry ice and subsequently lysed with TRIZol (Invitrogen Life Technologies, Carlsbad, CA), and total cellular RNA was isolated, per the manufacturer’s protocol. All experiments have been performed a minimum of three times, and representative data are presented.

RNase protection assays (RPA)

Total cellular RNA was isolated from lung or splenocytes using TRizol reagent. Multiprobe RPA kits were purchased (BD Pharmingen), and 10 μg of RNA from each sample was hybridized with 32P-labeled probe sets (mCK1, mCK5, or mCR5) and the RPA was completed, according to the manufacturer’s protocol. The products were separated on a 5% polyacrylamide gel, subsequently detected by exposure to x-ray film, and also analyzed on a phosphor imager (Molecular Imager; Bio-Rad, Hercules, CA); the signal intensity was determined using MultiAnalyst software (Bio-Rad). The ratio of the signal intensity of the gene of interest to GAPDH was determined, and the mean ± the SD of replicate samples was determined and presented.

Proliferation assays

Splenocytes were isolated from wild-type or CD28-deficient mice in the BALB/c background following immunization with OVA protein, as described above. A total of 5 × 10^5 cells per well was plated in each well of a round-bottom 96-well plate, and the cells were stimulated with graded doses of OVA or with a combination of PMA (5 ng/ml) and ionomycin (0.1 μg/ml). In the indicated experiments, splenocytes were first activated and Th2 differentiated for 7 days, as described above. The cells were then washed with fresh medium and rested overnight in the absence of Ag or exogenous cytokine. The cells were then restimulated with OVA 233–339 peptide for 48 h. Proliferation was determined by pulsed with 1 μCi of tritiated thymidine for the final 8 h of the culture period; the cells were subsequently harvested onto glass microfiber filters; and incorporated tritium was determined by liquid scintillography.

Eosinophil recruitment to the peritoneum

Mice were injected i.p. with 4 mg of Sephadex G200 (Amersham Biosciences, Uppsala, Sweden) in 2 ml of sterile PBS, as previously described (18). Twenty-four hours later, the peritoneal cavity was lavaged with sterile PBS, and cell counts and differential analysis were performed.

Statistics

All analysis was performed using two-tailed independent t test with Microsoft Excel software.

Results

Lack of T cell priming in CD28-deficient mice

Work from several laboratories has demonstrated that costimulation through CD28 is required for the generation of allergic airway inflammation (3–6). We had previously shown that CD28-deficient C57BL/6J mice failed to respond to sensitization and inhaled challenge with OVA (6). To examine effector cell function using adoptive transfer studies, we needed to use mice in the BALB/c background, as this is the genetic background of the DO11.10 OVA TCR transgenic mouse. Therefore, we first confirmed that BALB/c CD28−/− mice exhibited a similar phenotype as C57BL/6J CD28−/− mice. Wild-type or CD28-deficient BALB/c mice were immunized and challenged with OVA, as previously described (6). Tissue was harvested 72 h following challenge, and lung sections were examined histologically for the presence of inflamed cells. Eosinophils were specifically detected by assaying for the presence of cyanide-resistant peroxidase activity on frozen tissue sections. As shown in Fig. 1A, wild-type BALB/c mice develop peribronchial and perivasculary inflammatory cell infiltrates characterized by the presence of eosinophils. Consistent with our earlier work, the CD28-deficient mice had minimal inflammatory cell infiltrate and failed to recruit eosinophils to the lung.

The lack of an intrapulmonary response to inhaled challenge might be due to problems with initial T cell Ag recognition and clonal expansion (priming), or due to defects in subsequent stages of the immune response. Effective priming results in an increase in the precursor frequency of Ag-specific T cells, such that upon in vitro restimulation, a measurable increase in proliferative response can be observed. We tested whether there were differences in priming following immunization with OVA in wild-type or CD28-deficient mice. Splenocytes from mice that had been immunized with either OVA/alum or alum alone were isolated and restimulated with OVA in vitro (Fig. 1B). Cells isolated from wild-type mice immunized with OVA/alum demonstrated a dose-dependent increase in proliferation in response to OVA. The proliferation observed in cells isolated from CD28-deficient mice was not different from control mice that had not been immunized with OVA, suggesting that there was not effective priming. The total number and percentage of CD4+ T cells in the spleen were not different between the wild-type and CD28-deficient mice (9, 19) (data not shown). Proliferation induced by pharmacologic activation with PMA and ionomycin was similar in all groups (data not shown). These data confirm a role for CD28 in the initial priming of T cells to Ag in vivo.

Transfer of in vitro activated and differentiated T cells demonstrates a requirement for CD28 in effector responses

The observation that CD28-deficient mice exhibited a defect in the initial priming to Ag did not exclude a role for CD28 in subsequent effector phases of the immune response. To examine this, we performed adoptive transfer studies using in vitro activated T cells isolated from wild-type or CD28-deficient DO11.10 mice. These cells were ideally suited as they express a TCR transgene specific for OVA 233–339. The cells were activated in vitro and skewed toward Th2 differentiation by the addition of exogenous IL-4 and anti-IL-12, as previously described (15, 16). These cells were then injected i.v. into naive CD28−/− or CD28−/− BALB/c mice. The following day, the mice were given an inhaled challenge of OVA and lung and BAL specimens were collected for analysis 72 h later.

Our previous studies demonstrated that in vivo Th2 differentiation following immunization and challenge with OVA did not occur in CD28-deficient mice (6). Thus, we determined whether in vitro differentiated CD28-deficient T cells exhibited a similar pattern of gene expression as wild-type cells. Intracellular cytokine

Abbreviations used in this paper: BAL, bronchoalveolar lavage; RPA, RNase protection assay.
staining performed 7 days following the initial activation under Th2 conditions confirmed that similar percentages of cells expressed IL-4 in cultures from wild-type or CD28-deficient mice (Fig. 2A). To further assess the phenotype of these cells, we performed a multiprobe RPA to examine the mRNA levels of several different cytokines, chemokines, and chemokine receptors (Fig. 2, B and C). Wild-type and CD28-deficient T cells had a similar pattern of cytokine expression, although both IL-5 and IL-13 mRNA levels were consistently elevated in samples from CD28-deficient mice. Chemokine expression was similar between groups, although MCP-1 levels tended to be decreased in samples from CD28-deficient mice. Expression of CCR1 and CCR5 was somewhat increased in CD28-deficient cells relative to wild type, although the difference was not great (Fig. 2C). Consistent with previously published data, proliferation of CD28-deficient T cells was diminished; however, those stimulated under Th2 conditions had a proliferative response equivalent to wild-type cells (Fig. 2D).

We reasoned that if the failure of CD28-deficient mice to develop allergic airway inflammation was due only to ineffective T cell priming, then transfer of wild-type T cells should restore the response of CD28-deficient mice. Similarly, we hypothesized that in vitro activation and differentiation of CD28-deficient T cells should render them capable of mediating inflammation in vivo. OVA-specific T cells that had been in vitro activated and differentiated were injected into naive wild-type or CD28-deficient BALB/c mice. The mice were subsequently given an inhaled challenge with OVA, and samples were collected for analysis 72 h later. Examination of H&E-stained tissue sections demonstrated that transfer of wild-type cells into either wild-type or CD28-deficient recipients led to peribronchial and perivascular inflammatory cell infiltration following inhaled Ag challenge (Fig. 3A, I and 2). In contrast, transfer of CD28-deficient T cells did not result in inflammation in either wild-type or CD28-deficient recipients (Fig. 3A, 3 and 4). Abundant eosinophils were observed when wild-type cells were transferred into wild-type recipients, but markedly less were noted in the lungs of CD28-deficient mice (Fig. 3A, 5–8). There was no inflammation observed in specimens obtained from mice that received cells, but no challenge or inhaled challenge without prior cell transfer (data not shown).

Analysis of the BAL fluid revealed no significant differences in total cell recovery in each experimental group, but essentially no eosinophils were recovered from the lungs of mice receiving CD28-deficient T cells (Fig. 3, B–D). In addition, there was a consistent decrease in the number and percentage of eosinophils recovered from the lungs of CD28-deficient mice that had received wild-type cells, although this did not quite reach statistical significance when analyzed by eosinophil number (p = 0.06), but was significant as a percentage of total cells (p < 0.01). Therefore, these cells did not fully correct for the absence of CD28 in the recipient mice, suggesting a requirement for CD28 on the host immune and/or nonimmune cells. There was a modest reduction in the total number of CD4 cells recruited to the lung in wild-type mice that had received CD28-deficient cells or CD28-deficient mice that had received wild-type cells, as detected by flow cytometric analysis of the BAL. However, there was a marked decrease in the number of CD4 cells present in the BAL when both donor and recipient lacked CD28 (Fig. 3E). Similar numbers and percentages of the transferred transgenic T cells were recovered in the BAL of each group, as determined by staining of the BAL cells with the clonotypic mAb KJ1–26 and flow cytometry, demonstrating that the differences were not due to the inability of Ag-specific CD28-deficient T cells to migrate to the lung (Fig. 3E and Table I).

**Reduced cytokine and chemokine gene expression in vivo induced by effector T cells lacking CD28**

The adoptive transfer studies suggested that CD28 was important for T cell function beyond initial clonal expansion and differentiation. Analysis of whole lung RNA from recipient mice revealed that mice that had received CD28+ T cells in transfer had similar chemokine and cytokine profiles, despite differences in the inflammatory cell infiltrate (Fig. 4, A, B, D, and E). In contrast, very little cytokine or chemokine mRNA was detected in samples from mice that received CD28-deficient cells in transfer. Both IL-15 and
RANTES levels were comparable between groups, demonstrating that not all mediator mRNA levels were altered.

As we had noted a reproducible decrease in the number of eosinophils recruited to the lungs of CD28-deficient recipient mice, we directly examined the level of IL-13 and eotaxin protein present in the BAL, as these mediators are particularly important in the allergic response in the lung and in the recruitment of eosinophils (20–23). There was no significant difference in the level of these mediators in the BAL of CD28+/+ or CD28−/− mice that had received CD28+/+ cells in transfer (Fig. 4). Thus, a deficiency in these mediators in the lung does not account for the decrease in eosinophil recruitment.

CD28 regulates proliferation and IL-4 secretion on restimulation of activated T cells in vitro

The failure of CD28-deficient Th2 cells to mediate inflammation in vivo despite normal expression of cytokines following primary
stimulation suggested impairment in secondary responses. To directly examine this, we restimulated activated Th2 cells in vitro with either Ag alone, or in combination with anti-CD28 to provide optimal costimulation, or CTLA4Ig to block any endogenous B7-1 and B7-2, and measured proliferation and IL-4 secretion (Fig. 5, A and B). Both were reduced in cultures of cells lacking CD28, particularly at lower concentrations of Ag, thus demonstrating that CD28 functions to augment these functions in previously activated T cells. The reduction in secondary cytokine secretion and cell expansion upon restimulation may explain the failure of the transferred CD28-deficient Th2 cells to mediate allergic airway inflammation. Similarly, the failure of wild-type cells to fully reconstitute CD28-deficient hosts may be due to the poor intrinsic response of the endogenous Ag-nonspecific T cell population that is corecruited to the lung.

**CD28-deficient mice have impaired recruitment of eosinophils**

Despite similar levels of cytokine expression in the lung, as determined by examination of both mRNA and protein levels, transfer of wild-type T cells into CD28-deficient recipients failed to fully restore the wild-type phenotype following allergen challenge. Specifically, we observed a decrease in the recruitment of eosinophils to the lung. Although lung and BAL cytokine levels appeared similar to control animals, it remained possible that systemic levels of cytokine were inadequate to promote eosinophil maturation and recruitment to the lung. To address this question,
we administered systemic IL-5 to CD28-deficient mice that had received wild-type, OVA-specific Th2 cells in transfer. We chose IL-5, as it has been shown to be critical in the maturation and survival of eosinophils (24). Injection of IL-5 failed to restore eosinophil recruitment to the lung following allergen challenge, although it is possible that we were unable to achieve sufficient

Table I. Quantification of CD4 and Ag-specific T cells

<table>
<thead>
<tr>
<th>Donor-Recipient</th>
<th>CD4+ T cells</th>
<th>Transgenic T cells</th>
</tr>
</thead>
<tbody>
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<td>CD28+/+CD28+/+</td>
<td>6.04 × 10^4 ± 2.70 × 10^4</td>
<td>2.09 × 10^4 ± 1.36 × 10^4</td>
</tr>
<tr>
<td>CD28−/−CD28+/+</td>
<td>2.84 × 10^4 ± 1.39 × 10^4</td>
<td>9.54 × 10^3 ± 5.81 × 10^3</td>
</tr>
<tr>
<td>CD28+/+CD28−/−</td>
<td>3.45 × 10^4 ± 1.79 × 10^4</td>
<td>4.89 × 10^4 ± 1.66 × 10^4</td>
</tr>
<tr>
<td>CD28−/−CD28−/−</td>
<td>7.51 × 10^3 ± 2.08 × 10^3</td>
<td>6.82 × 10^2 ± 8.80 × 10^2</td>
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* Cells recovered in the BAL were counted and stained with mAb against CD4 and the transgenic TCR (using KJ1–26) and analyzed by flow cytometry. The total number of CD4+ and transgenic TCR+ cells was calculated.

**FIGURE 4.** Expression of chemokines and cytokines at the site of Ag challenge. Total cellular RNA was isolated from lungs of mice that had received either CD28+/+ or CD28−/− OVA-specific Th2 cells, followed by inhaled Ag challenge. A, Expression of mRNA for specific cytokines was determined by multiprobe RPA. Shown is one representative experiment. Each lane represents an individual mouse. B, The signal intensity for each band was determined on a phosphor imager, and each sample was normalized to the intensity of the GAPDH band. The mean and SD of three replicate samples obtained from individual mice is presented graphically. C, Levels of IL-13 in the BAL fluid were measured by ELISA. Presented is the mean ± the SD of six independent samples. D, Representative multiprobe RPA analysis for chemokine expression of whole lung prepared as described for A. E, Quantification of chemokine expression from lungs of mice as described for B. F, Levels of eotaxin in the BAL fluid were measured by ELISA. Presented is the mean and SD of six independent samples. **, p < 0.01 in comparison with wild-type cells transferred into wild-type recipients.
In the absence of CD28, CD28<sup>−/−</sup> or CD28<sup>+/−</sup> DO11.10 splenocytes were activated with OVA<sub>323-339</sub> (0.3 μM) peptide in the presence of exogenous IL-4 and anti-IL-12. After 7 days of culture, the cells were restimulated with OVA<sub>323-339</sub> alone, plus anti-CD28 (1 μg/ml) or CTLA4Ig (10 μg/ml) for 48 h, and proliferation (A) and IL-4 (B) secretion were determined. Shown is the mean result obtained from three individual mice of each genotype. Each measurement was done in either quadruplicate (proliferation) or triplicate (IL-4) for each mouse.

**FIGURE 5.** Impaired secondary cytokine and proliferative responses in the absence of CD28. CD28<sup>+/−</sup> or CD28<sup>−/−</sup> DO11.10 splenocytes were activated with OVA<sub>323-339</sub> (0.3 μM) peptide in the presence of exogenous IL-4 and anti-IL-12. After 7 days of culture, the cells were washed and rested overnight in fresh medium with no additional Ag or cytokines. The cells were then restimulated with OVA<sub>323-339</sub> alone, plus anti-CD28 (1 μg/ml) or CTLA4Ig (10 μg/ml) for 48 h, and proliferation (A) and IL-4 (B) secretion were determined. Shown is the mean result obtained from three individual mice of each genotype. Each measurement was done in either quadruplicate (proliferation) or triplicate (IL-4) for each mouse.

A further possibility to explain the failure to recruit eosinophils to the lung of CD28-deficient mice following transfer of activated, Ag-specific Th2 cells is that there is an intrinsic defect in the host eosinophils. Previous work has demonstrated expression of both CD28 and B7 molecules on human eosinophils, raising the possibility that the lack of CD28 expression directly affected eosinophil development, survival, and/or function (25,26). However, in contrast to the published human studies, we were unable to detect CD28 on either resting or cytokine-stimulated murine eosinophils (data not shown). However, we did note a consistent decrease in the recruitment of eosinophils to the peritoneum following i.p. injection with Sephadex (Fig. 6). Both the percentage and number of eosinophils were reduced in peritoneal exudate cells elicited by Sephadex. This was not explained by decreased precursors in the marrow or decreased peripheral blood eosinophil counts (data not shown). Thus, the defect in eosinophil recruitment is not limited to intrapulmonary responses, but may represent a more generalized problem with eosinophil function in mice lacking CD28.

**FIGURE 6.** Peritoneal eosinophil recruitment is decreased in CD28-deficient mice. Wild-type BALB/c or CD28-deficient mice were injected i.p. with 4 mg of Sephadex G200, and 24 h later peritoneal exudate cells were collected. Cell counts and differential analysis revealed a decrease in both the absolute number and percentage of eosinophils recruited to the peritoneal cavity. n = 6 mice of each genotype examined.

**Discussion**

Atopic asthma is characterized by a Th2-mediated immune response in the lung. Upon exposure to Ag, sensitized individuals develop an acute inflammatory response that includes the recruitment of T cells and eosinophils to the airway. The elaboration of inflammatory mediators is thought to contribute to the acute bronchoconstriction that is a hallmark of asthma, as well as airway remodeling that may occur in the setting of chronic disease.

T cells are central to the pathogenesis of asthma. Via elaboration of specific mediators, activated T cells coordinate the recruitment and function of inflammatory cells. The T cell response to Ag is dependent upon whether costimulatory receptors such as CD28 are also engaged (27). Concomitant signaling through CD28 leads to augmented proliferation and cytokine production (1). Interference with this pathway in vivo can influence inflammatory responses. Specifically, blockade of CD28 has been shown to prevent airway inflammation and hyperreactivity in a murine model of atopic asthma (3–6). This effect might be due to failure of the T cell to respond to the initial sensitization with Ag, or alternatively, it may be secondary to altered effector responses at the site of inflammation. We performed a series of experiments designed to determine whether inhibition of CD28 prevented allergic airway inflammation solely by preventing the initial sensitization of the T cell, or whether subsequent effector responses were also modulated by CD28. Our data support a role for CD28 in both phases of the response.

Examination of T cell responses following systemic immunization with OVA demonstrated impaired priming. However, this result did not exclude a requirement for CD28 in subsequent effector responses. Transfer of in vitro activated and Th2-differentiated T cells into naive recipient mice subsequently given an inhaled Ag challenge allowed us to examine the requirement for CD28 in the effector phase. Although transfer of wild-type Th2 cells into CD28-deficient recipients effectively restored most aspects of the inflammatory response, CD28-deficient Th2 cells failed to mediate inflammation when transferred into wild-type or CD28-deficient hosts. This was due to a failure of the T cells to differentiate into Th2 cells in vitro, as the cytokine profile of the CD28-deficient T cells was similar to that of wild-type cells, and in fact even demonstrated increased expression of some Th2 cytokines. There were some differences in expression of chemokine and chemokine receptor expression noted in the CD28-deficient cells, including a decrease of MCP-1 expression. This is of potential importance, as
this chemokine has been described in specimens from asthmatic patients (28, 29). Further evidence supporting a role for this chemokine in allergic asthma comes from animal studies, in which neutralization of MCP-1 attenuated airway hyperreactivity in response to allergen (30). However, direct administration of the murine homolog of MCP-1, JE/CCL2, did not restore eosinophil recruitment (S. L. Kimzey and J. M. Green, unpublished observations).

Although there was a change in the pattern of expression of some chemokine receptors on CD28-deficient cells, we felt it unlikely that this accounted for the inability of CD28-deficient T cells to mediate inflammation. If mechanistically significant, altered chemokine receptor expression would be expected to result in changes in recruitment of the transferred cells to the lung. However, we did not observe any significant difference in the number or percentage of KJ1–26-positive T cells in the BAL fluid. There was a trend toward a decrease in the number of bystander CD4+ cells recruited if either the donor or recipient cells lacked CD28, but this only became statistically significant if both donor and recipient were CD28 deficient. Decreased bystander cell recruitment or activation could potentially limit the inflammatory response, particularly in CD28-deficient hosts, thus accounting for some of the observations.

In vitro analysis demonstrated that CD28-deficient T cells had a significant reduction in both proliferation and IL-4 secretion upon secondary stimulation with Ag (Fig. 5). These data suggested that upon re-exposure to Ag in vivo, the transferred CD28-deficient Th2 cells were unable to sustain sufficient cytokine secretion to mediate an inflammatory response. In support of this, little cytokine mRNA was detected in the lung specimens isolated from mice that received CD28-deficient T cells in transfer.

Wild-type cells transferred into CD28-deficient mice resulted in airway inflammation. However, we observed a consistent decrease in the number and percentage of eosinophils recovered in the BAL fluid. We hypothesized that this might be due to a decrease in specific mediators that recruit eosinophils, particularly as the majority of T cells recruited to the lung are bystander, host T cells that lack CD28. However, we observed no significant difference in the expression of multiple cytokine or chemokine genes in the lung, as assessed by RPA when compared with transfer into wild-type mice. In addition, direct measurement of IL-13 and eotaxin protein levels in the BAL fluid revealed no difference between the two groups receiving wild-type T cells. Nonetheless, it remained a possibility that systemic cytokine levels were decreased in CD28-deficient recipients. IL-5 has been shown to be important in the maturation and mobilization of eosinophils from the marrow (31, 32). Therefore, we reasoned that undetected decreases in systemic or local IL-5 levels might account for the failure to recruit eosinophils. Systemic administration of exogenous IL-5 was not sufficient to restore eosinophil recruitment, suggesting that other factors were needed, or that we were unable to achieve adequate concentrations in vivo.

Recently, CD28 has been described on human eosinophils (25, 26). Ligation of CD28 on eosinophils resulted in release of cytokines, including IL-13, IL-2, and IFN-γ. The presence of CD28 on eosinophils raises the possibility that it is important in the development or function of these cells. Therefore, the decreased eosinophil recruitment observed in CD28-deficient recipients receiving wild-type T cells might be due to intrinsic defects in the eosinophils of the recipient mice. There was no detectable difference in the number of eosinophils or precursors in the bone marrow or peripheral blood of CD28-deficient mice. Furthermore, we were unable to detect expression of CD28 on murine eosinophils, either in a resting state, or following culture with IL-3, IL-5, GM-CSF, or activation with PMA and ionomycin. However, we did observe in CD28-deficient mice a consistent decrease in eosinophil recruitment to the peritoneal cavity following Sephadex injection. Thus, it remains a possibility that there is a more global defect in eosinophil function following development in a CD28-deficient background.

Although the role of CD28 in the initial activation of naïve T cells has been well described, less importance has been placed on its role in subsequent phases of T cell function. The data presented support a role for CD28 in mediating an allergic inflammatory response through a mechanism beyond impaired T cell priming. Given that effective reagents exist for inhibiting CD28 ligation in vivo, therapy directed at this may be of use in individuals that have already been sensitized to specific Ags.

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References


