Brief Definitive Report

β2-microglobulin–dependent T Cells Are Dispensable for Allergen-induced T Helper 2 Responses

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Summary

CD4+ and CD8+ α/β+ T cells of the T helper cell (Th)2 phenotype produce the cytokines IL-4, IL-5, and IL-13 that promote IgE production and eosinophilic inflammation. IL-4 may play an important role in mediating the differentiation of antigenically naive α/β+ T cells into Th2 cells. Murine NK1.1+ (CD4+ or CD4−CD8−) α/β+ T cells comprise a β2-microglobulin (β2m)–dependent cell population that rapidly produces IL-4 after cell activation in vitro and in vivo and has been proposed as a source of IL-4 for Th2 cell differentiation. α/β+ CD8+ T cells, most of which require β2m for their development, have also been proposed as positive regulators of allergen-induced Th2 responses. We tested whether β2m–dependent T cells were essential for Th2 cell-mediated allergic reactions by treating wild-type, β2m-deficient (β2m−/−), and IL-4-deficient (IL-4−/−) mice of the C57BL/6 genetic background with ovalbumin (OVA), using a protocol that induces robust allergic pulmonary disease in wild-type mice. OVA-treated β2m−/− mice had circulating levels of total and OVA-specific IgE, pulmonary eosinophilia, and expression of IL-4, IL-5, and IL-13 mRNA in bronchial lymph node tissue similar to that of OVA-treated wild-type mice. In contrast, these responses in OVA-treated IL-4−/− mice were all either undetectable or markedly reduced compared with wild-type mice, confirming that IL-4 was required in this allergic model. These results indicate that the NK1.1+ α/β+ T cell population, as well as other β2m–dependent populations, such as most peripheral α/β+ CD8+ T cells, are dispensable for the Th2 pulmonary response to protein allergens.

Cytokines produced by T cells are critical regulators of the immune response to pathogens and antigens (1, 2). CD4+ effector and memory T cells that develop during chronic immune responses tend to express cytokines in mutually exclusive patterns of either the T helper cell (Th)1 (IL-2, IFN-γ, and TNF-β) or the Th2 (IL-4, IL-5, and IL-13) phenotypes (1, 2). Coordinate production of Th2 cytokines is characteristic of responses either to infection with metazoan parasites (2) or to repeated allergen exposure (3). The consequences of Th2 cytokine expression include increased production of IgE by B cells, an IL-4–dependent effect (4) that, at least in humans, may be augmented by IL-13 (3), as well as inflammation of tissues with eosinophils, a feature that is IL-5 dependent (5–7). Recent studies also suggest that α/β+ CD8+ T cells are a potential source of Th2 cytokines, such as IL-4 and IL-5 (1, 8).

Several lines of evidence suggest that IL-4 itself plays a central role in Th2 cell development in vivo. First, mice deficient in IL-4, as a result of disruptive gene targeting, fail to develop IL-5–producing T cells or eosinophilia after challenges with helminths or allergens (9, 10). Second, treatment of wild-type mice with neutralizing antibodies against IL-4 during primary immunization with protein antigen reduces subsequent antigen–specific production of IL-4 by T cells ex vivo and IL-4 mRNA expression by splenocytes in vivo (11, 12). Third, studies of the differentiation of antigenically naive T cells in vitro demonstrate that IL-4 promotes Th2-like differentiation (13).

Since IL-4 production by antigenically naive T cells in response to conventional protein antigens or polyclonal activators tends to be low (14), other cellular sources of IL-4 production have been proposed to promote Th2 differentiation. These include non–T-lineage cells, such as mast cells and basophils (15), as well as cells of T-lineage, such as the murine NK1.1+ α/β+ T cell population (2, 16). NK1.1+ α/β+ T cells are either CD4+ or CD4−CD8− by their cell surface phenotype (16) and have been shown to rapidly produce relatively high levels of IL-4 after activation with anti-CD3 monoclonal antibody treatment in vivo (17). Murine NK1.1+ T cells require β2-microglobulin (β2m)–expressing hematopoietic cells for their development in vivo (18), and the T cell receptors of at least some of these cells recognize antigens bound to CD1, a nonclassic MHC molecule that associates with β2m on the cell surface (16).
Recently, it has been shown that β2m−/− mice, which lack the NK1.1+ T-lineage cell population, have an impaired ability to produce IgE in response to polyclonal activation in vivo induced by anti-CD3 mAb or goat anti-mouse IgD serum (19). However, it remains unclear whether NK1.1+ T cells are essential for IgE and other aspects of Th2 immunity induced by conventional protein antigens or if other T cells are important in this context, such as α/β+ CD8+ T cells.

Using a murine model of OVA-induced allergic pulmonary disease that requires IL-4 production, we show here that β2m-dependent T cell populations are dispensable for a robust Th2 immune response, including antigen-specific and total IgE expression, tissue eosinophilia, and increased expression of Th2 cytokines in lung-associated peripheral lymphoid tissue. These results suggest that neither NK1.1+ α/β+ T cells nor conventional α/β+ CD8+ T cells play a critical role in directing Th2 cell differentiation in vivo and that other cell types may provide sufficient IL-4 for this process.

Materials and Methods

Animals. Wild-type C57BL/6j mice were obtained from Jackson Laboratories (Bar Harbor, ME), β2m−/− mice homozygous for targeted disruption of the β2m gene (20) and congenic on the C57BL/6j background were generously provided by Dr. Christopher Wilson (University of Washington). These mice were confirmed to be homozygous for the mutant β2m allele by PCR with primers specific for the targeted disruption of the 132m gene (20) and confirmed to be homozygous for targeted disruption of the IL-4 gene (4) were obtained from Dr. B.J. Fowlkes (National Institutes of Health, Bethesda, MD) and were used after 10 generations of backcrossing onto a C57BL/6j background. All mice were housed in a specific-pathogen-free facility and were 8-12 weeks old.

Experimental Protocol for the Induction of Allergic Pulmonary Disease. OVA-treated mice received single intraperitoneal injections of 100 μg of alum-precipitated crystalline OVA (Pierce, Rockford, IL) in 0.2 ml of normal saline on day 1 and day 14, and single intranasal doses of 50 μg of OVA in 50 μl of normal saline on day 14, day 25, day 26, and day 27 as described previously (21). Sham-sensitized mice were treated identically to OVA-treated mice except that intraperitoneal injections consisted of 100 μg of alum in normal saline, and intranasal doses consisted of 50 μl of normal saline. Mice were euthanized on day 28, and plasma, bronchoalveolar lavage fluid, and bronchial lymph node tissue were collected.

Table 1. Plasma Total and OVA-specific IgE Levels in Wild-type, β2m−/−, and IL-4−/− Mice

| Mouse Strain | Total IgE (ng/ml) | OVA-specific IgE (U/ml) |
|--------------|------------------|------------------------|
| Wild-type, OVA (n = 11) | 1144 ± 368* | 7.2 ± 0.9* |
| β2m−/−, OVA (n = 12) | 1536 ± 424* | 6.5 ± 1.1* |
| IL-4−/−, OVA (n = 11) | 16 ± 0 | <0.1 |
| Wild-type, sham (n = 8) | 64 ± 2 | <0.1 |
| β2m−/−, sham (n = 5) | 160 ± 64 | <0.1 |

Mean ± SEM are shown. The data are representative of two independent experiments. There were no significant differences in total or OVA-specific IgE levels between wild-type mice and β2m−/− mice after OVA treatment.

Results and Discussion

To determine the importance of β2m-dependent T cells for the development of Th2 responses in vivo, β2m−/−, and wild-type C57BL/6j mice were treated with OVA by a

Plasma Total and OVA-specific IgE Levels in Wild-type, β2m−/−, and IL-4−/− Mice

* P <0.05 for wild-type or β2m−/− mice after OVA treatment versus all other groups by ANOVA.
protocol that reliably induces OVA-specific IgE, eosinophil accumulation in the lung parenchyma and airway, and increased expression of Th2 cytokines in bronchial lymph nodes from mice of the C57BL/6 strain (21). OVA-treated wild-type mice had >10-fold higher mean plasma levels of total IgE than sham-sensitized wild-type animals (Table 1). As expected, OVA-specific IgE was demonstrable in the plasma of OVA-treated wild-type mice but was undetectable in samples from sham-sensitized wild-type mice (Table 1). The plasma levels of total and OVA-specific IgE in OVA-treated fl2m -/-/mice were similar to those of OVA-treated wild-type animals (Table 1), indicating that IgE production was unperturbed.

Our results differ from those of Yoshimoto et al. (19), who found markedly lower total IgE levels in fl2m -/-/mice compared with wild-type C57BL/6 mice 9 d after injection with goat anti-mouse IgD serum. In these experiments, the IgE response of fl2m -/-/mice to anti-IgD injection as well as the NK1.1 + T cell population was reconstituted to wild-type levels by adoptive transfer of NK1.1 + thymocytes and T cell-depleted splenocytes from wild-type animals (19). There are at least three possible explanations why the induction of IgE by anti-IgD treatment may require NK1.1 + T cells, whereas immunization with protein antigen does not. First, NK1.1 + T cells might be needed for IgE production in situations in which B cells are the principal APC for T cells, as appears to be the case for anti-IgD treatment (23), but might be dispensable for IgE produced after immunization with conventional protein antigens, a situation in which other APC, particularly dendritic cells, are likely to be important (24). Second, it is possible that IgE induced after primary immunization with protein antigen may be dependent, at least in part, on NK1.1 + T cells, but that this requirement is abrogated with secondary and additional immunizations. However, we do not favor this possibility since fl2m -/-/ and wild-type mice had similar levels of OVA-specific IgE 9 d after a single intraperitoneal injection of alum-precipitated OVA (the OVA-specific IgE titer [mean ± SEM] in fl2m -/-/mice [n = 5] and wild-type mice [n = 4] was 2.33 ± 0.97 U/ml and 1.86 ± 0.44 U/ml, respectively; P = 0.7 by the two-tailed, unpaired Student's t test). A third possibility is that during development in fl2m -/-/mice, another subpopulation of T cells is able to produce IL-4, and to compensate for the loss of NK1.1 + T cells or other fl2m-dependent T cells.

We analyzed BAL fluid obtained on day 28 of OVA treatment to determine if the pulmonary leukocyte inflammatory response was altered in fl2m -/-/mice compared with wild-type mice. In both fl2m -/-/ and wild-type mice, OVA treatment resulted in a significantly greater total number of cells in the BAL fluid compared with that of sham sensitization (Table 2), with eosinophils comprising the majority of cells (Table 2 and Fig. 1, A and B). Eosinophils were not observed in sham-sensitized controls of either

### Table 2. Bronchoalveolar Lavage Fluid Leukocytes in Wild-type, fl2m -/-, and IL-4 -/- Mice

| Mouse Strain and Treatment | Cell Number (×10⁴/ml) | Percentage of Eosinophils |
|---------------------------|-----------------------|---------------------------|
| Wild-type, OVA (n = 11)   | 98.4 ± 31.1*          | 66.8 ± 1.8*               |
| fl2m -/-, OVA (n = 12)    | 111.4 ± 17.9*         | 65.5 ± 2.2*               |
| IL-4 -/-, OVA (n = 11)    | 18.1 ± 2.3            | 2.0 ± 0.9                 |
| Wild-type, sham (n = 8)   | 7.0 ± 0.9             | <0.1                      |
| fl2m -/-, sham (n = 5)    | 7.6 ± 1.7             | <0.1                      |

Mean ± SEM are shown. The data are representative of two independent experiments. There were no significant differences between wild-type mice and fl2m -/-/mice after OVA treatment.

*P <0.05 for wild-type or fl2m -/-/mice after OVA treatment versus all other groups by ANOVA.
the \(\beta_2m^{-/-}\) or wild-type groups (Table 1 and Fig. 1 C). Furthermore, a marked eosinophilic infiltrate in the pulmonary parenchyma was also observed for \(\beta_2m^{-/-}\) and wild-type mice after OVA treatment, but not in sham-sensitized animals (data not shown).

Previous work using mice has shown that eosinophil accumulation in the lung and airway in response to OVA treatment requires the Th2 cytokines, IL-4 (10) and IL-5 (5), and is associated with locally increased expression of these cytokines (7). To determine if the eosinophilic pulmonary inflammation in \(\beta_2m^{-/-}\) and wild-type mice was accompanied by locally increased production of Th2 cytokines by lymphocytes, the amount of cytokine transcripts in the draining bronchial lymph nodes from individual animals was analyzed by reverse transcriptase (RT)-PCR (Fig. 2). The bronchial lymph nodes of wild-type mice or \(\beta_2m^{-/-}\) mice treated with OVA (Fig. 2 A, lanes 4–7 and lanes 10–13, respectively) had substantially higher levels of transcripts for IL-4, IL-5, and IL-13 compared with those from sham-sensitized controls (lanes 1–3, 8, 9). Cytokine mRNA levels in wild-type or \(\beta_2m^{-/-}\) mice were low to undetectable after sham sensitization (Fig. 2 A). These higher cytokine levels in OVA-treated samples were not attributable to an increased amount of total RNA that was amplifiable by PCR, since the ratio of the RNA-derived and competitor-derived products for HPRT, an abundant housekeeping gene, was similar in all samples. The amount of competitor-derived IL-4 products was also similar in all lanes, indicating that the overall efficiency of PCR amplifications did not vary significantly from sample to sample. The increase in Th2 cytokine abundance in bronchial lymph node tissue suggested that activation of T cells in vivo by allergen occurred normally in \(\beta_2m^{-/-}\) mice. Consistent with this idea, we also found that bronchial lymph node cells from OVA-treated \(\beta_2m^{-/-}\) or wild-type mice had similar levels of OVA-specific cell proliferation in vitro (data not shown). Together, these data indicate that T cell priming by antigen and expression of Th2-type cytokines occurred normally in \(\beta_2m^{-/-}\) mice. Our results are consistent with those of Guery et al. (25), who found that continuous administration of low doses of soluble protein antigens to either wild-type or \(\beta_2m^{-/-}\) BALB/c mice resulted in Th2-like cells that preferentially produced IL-4, rather than IFN-\(\gamma\), after stimulation with antigen ex vivo. Our results extend these observations by demonstrating that \(\beta_2m\)-dependent T cell populations are dispensable in C57BL/6 strain mice for multiple in vivo events characteristic of the allergic response.
To confirm that the IgE production, eosinophilic inflammation, and Th2 cytokine production induced in wild-type and β2m−/− mice by our OVA-treatment protocol were IL-4 dependent, these responses were analyzed in IL-4−/− mice. As expected, based on earlier studies (4, 6), OVA-treated IL-4−/− mice had substantially decreased plasma levels of total and OVA-specific IgE compared with wild-type mice (Table 1). The low but detectable amount of total and OVA-specific IgE in IL-4−/− mice suggests that a portion of IgE production in vivo may be IL-4 independent and is consistent with a report of reduced but detectable levels of total IgE in IL-4−/− mice infected with Plasmodium (26). Both BAL fluid cellularity and eosinophilia (Table 2 and Fig. 1 D) were also markedly decreased in IL-4−/− mice compared with wild-type animals. Consistent with these findings, which suggested a decreased Th2 immune response in vivo, the levels of IL-5 and IL-13 mRNA in the bronchial lymph nodes of OVA-treated IL-4−/− mice were dramatically reduced compared with wild-type mice (Fig. 2 B). These results also indicate that IL-13 expression during allergic reactions in vivo is largely IL-4 dependent, at least in mice.

Our results also suggest that most conventional α/β+ CD8+ T cells are dispensable for the Th2 pulmonary responses to OVA. This contrasts with a report (27) in which treatment of thymectomized BALB/c mice with anti-CD8-α mAb prevented OVA-induced eosinophil accumulation and IL-5 mRNA expression by bronchial lymph nodes. Whether these different results reflect the strains used—C57BL/6 versus BALB/c—or the methods employed to eliminate CD8+ T cells—genetic versus thymectomy and antibody treatment—remains unresolved. It is possible that anti-CD8-α mAb treatment may eliminate mucosal CD8+ T cells that are required for the allergic pulmonary response, but which are not dependent on β2m for their development, such as CD8+ γ/δ+ T cells (28). Interestingly, a population of NK1.1+ γ/δ+ thymocytes is present in β2m−/− mice, and like NK1.1+ α/β+ T cells, these cells rapidly produce IL-4 after polyclonal activation (29). Therefore, peripheral T cells derived from this NK1.1+ γ/δ+ thymocyte population could provide IL-4 for Th2 cell differentiation in β2m−/− as well as wild-type mice. Whether this cell population expresses CD8-α in the periphery remains to be determined.

In summary, our results indicate that the Th2 allergic pulmonary response induced by OVA in this model is IL-4 dependent but independent of β2m expression. This argues that β2m-dependent α/β+ T cells, such as NK1.1+ α/β+ T cells, and most class I MHC-restricted α/β+ CD8 T cells are dispensable for a robust Th2 response to protein allergens in vivo. Further studies are required to determine the importance of other cellular sources of IL-4 proposed to influence Th2 differentiation, such as mast cells and basophils, conventional α/β+ T cells, or γ/δ+ T cells (2, 15).

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