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Cutting Edge: MHC Class II Expression by Pulmonary Nonhematopoietic Cells Plays a Critical Role in Controlling Local Inflammatory Responses

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The interaction of CD4+ T cells with MHC class II (MHCII)-expressing hematopoietic APCs plays a critical role in both the generation of protective immune responses and maintenance of tolerance in the lung. The functional significance of MHCII expression by nonhematopoietic stromal cells, however, has not been defined in vivo. Using a novel mouse model of orthotopic lung transplantation, we demonstrate that selective elimination of MHCII expression on nonhematopoietic cells leads to attenuation in tumor growth. Furthermore, in a clinically relevant model of pulmonary metastases, the elimination of MHCII on nonhematopoietic cells led to attenuation in tumor growth.

Materials and Methods

Animals

Male C57BL/6 (B6) and OT-II mice, TCR transgenic for OVA323–339, were purchased from The Jackson Laboratory (Bar Harbor, ME), whereas B6/SJL CD45.1+ and B6/129 F1 mice were obtained from Taconic Farms (Hudson, NY). MHCII-deficient mice on a B6 background (B6II−−) were originally developed by introducing a loss of functional mutation into the Aβ1 gene in animals that harbor a natural deletion in their Eα gene (8). B6Foxp3+ mice were provided by A.Y. Rudensky (Memorial Sloan-Kettering Cancer Center, New York, NY) (9). Lung and bone marrow transplants were performed as described previously (7, 10). Unless specifically identified, all mice were sacrificed 2 wk after transplantation. For tumor studies, mice were injected i.v. with 2.5 × 10^5 B16 murine melanoma (American Type Culture Collection, Manassas, VA) 1 wk after transplantation and euthanized 21 d later.

Flow cytometry

Tissues were processed as previously described (11) and stained with anti-I-A/I-E (clone M5/114.15.2), anti-CD90.2 (clone 30-H12), anti-CD4 (clone RM4-5), anti-CD8 (clone 53-6.7), anti-CD62L (clone MEL-14), anti-CD44 (clone IM7), anti-GITR (DTA-1), anti-CD45.1 (clone A20), anti-CD45.2 (clone 104), and isotype controls (BD Biosciences, Franklin Lakes, NJ). Human lung tissue was stained with anti-CD45 (clone HI30), anti-DR

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Abbreviations used in this paper: B6, C57BL/6; GITR, glucocorticoid-induced TNFR-related protein; MHCII, MHC class II; Treg, regulatory T cell.

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Results and Discussion

Mouse lung nonhematopoietic cells express MHCII constitutively

Similar to humans, a substantial portion of pulmonary nonhematopoietic cells, such as vascular endothelium and airway epithelium, express MHCII constitutively in the mouse (Fig. 1A, 1B). Furthermore, unlike the case for secondary lymphoid organs, up to 50% of all MHCII+ cells in the lung are nonhematopoietic in both mice and humans (Fig. 1C). Nonhematopoietic pulmonary cells also express the invariant chain (Fig. 1D) and are able to take up and process exogenous Ag, as evidenced by cleavage and fluorescence of DQ-OVA (Fig. 1E). Taken together with recent reports by our group and others that the lung provides a suitable environment for T cell activation (11, 13), these findings support the notion that CD4+ T cell-restricted Ag presentation by pulmonary nonhematopoietic cells may have functional consequences.

Although we and others have used donor organs derived from bone marrow chimeras to study CD4+ T cell allo-recognition in transplant rejection (10), the use of bone marrow chimeras as hosts to study MHCII expression on nonhematopoietic cells is limited by altered thymic CD4+ T cell development (14). To evaluate lung transplantation as a model for the study of CD4+ T cell-restricted Ag presentation, we engrafted left lungs of B6C3H/HeJ mice into B6 recipients and documented near complete substitution of donor with recipient CD4+ T cells and professional hematopoietic APCs (defined as CD45/Rag1− MHCII+) within a few days of engraftment (Fig. 1F). Nonhematopoietic cells of the graft, however, remained of the donor genotype (Fig. 1G). Thus, transplantation of a B6II− donor into a wild-type B6 recipient can be used to create a "chimeric lung" consisting of MHCII-deficient nonhematopoietic cells repopulated by wild-type CD4+ T cells and MHCII-expressing hematopoietic professional APCs. Such an experimental system would result in the local disruption of CD4+ T cell-restricted Ag presentation solely by nonhematopoietic cells in the lung.

Elimination of MHCII on pulmonary nonhematopoietic cells results in local inflammation

Two weeks after transplantation, B6 to B6 lung grafts remain free of inflammation, but B6II− grafts demonstrate inflammatory changes characterized by perivascular and peribronchial infiltrates (Fig. 2A) with an increase in the number of CD4+ T cells and a higher proportion of CD62Llo−CD44hi cells consistent with an effector memory phenotype (Fig. 2B). On the basis of the known heterogeneity of perivascular T cell infiltrates in pulmonary inflammation, we also analyzed CD8+ T cells and demonstrated increased accumulation and activation of this cell population as well (Fig. 2B) (12). No qualitative or quantitative differences are detectable in the CD4+ T cells in the mediastinal lymph nodes or native right lungs suggesting that differences between T cells in B6 and B6II− grafts are due to their local activation (Supplemental Fig. 1). Adoptive transfer of naive B665-6CD62Lhi−CD44loCD4+ T cells demonstrates increased generation of effector memory CD62Llo−CD44hi CD4+ T cells in the B6II− compared with B6 grafts with no detectable differences in the mediastinal lymph nodes (Fig. 2C) or native right lungs (data not shown). To rule out the possibility that the inflammatory changes were due to an alloimmune immune response to a
minor Ag in the B6II− mouse that may not be present in the B6 recipient, we performed single nucleotide polymorphism analysis of B6II− mice and determined that this mutant was 98.5% B6 origin (Supplemental Fig. 2A). To further rule out the possibility that minor Ags that have cosegregated with this deletion were contributing to the inflammation, we transplanted B6 and B6II− lungs into B6/B6II− F1 recipients and demonstrated inflammatory changes as well as increased T cell activation and accumulation only in B6II− grafts (Fig. 2D,2E). Similar differences in T cell activation and accumulation were evident if B6/129 F1 mice were used as graft recipients (Supplemental Fig. 2B). Furthermore, B6/B6II− F1 grafts did not elicit any inflammatory response when used as donors (Supplemental Fig. 2C). These data provided compelling evidence that inflammatory changes evident in B6II− lung grafts were a result of MHCII deficiency rather than a minor Ag mismatch. To determine whether the transient initial presence of hematopoietic MHCII− professional APCs in B6II− grafts contributes to the inflammatory response, we used donor organs derived from bone marrow chimeras, where MHCII expression was restricted to either the hematopoietic or nonhematopoietic cells. We found increased CD4+ T cell accumulation and activation only in mice lacking MHCII on nonhematopoic cells (Supplemental Fig. 2D).

Treatment of recipient mice with the CD4+ T cell-depleting Ab GK1.5 prior to transplantation eliminated differences in CD8+ T cell infiltration or activation (Fig. 2F). CD8+ T cells of animals treated with control rat IgG had a similar phenotype to unmanipulated mice depicted in Fig. 2B (data not shown), and the use of CD4+ T cell knockout recipients yielded results similar to those depicted in Fig. 2F. Collectively, these data suggest that MHCII expression on pulmonary nonhematopoietic cells plays a role in controlling T cell responses in a CD4+ T cell-dependent fashion.

On the basis of the finding that absence of MHCII on nonhematopoietic cells resulted in the activation of both CD4+...
and CD8+ T cells, as well as our previous in vitro data demonstrating the importance of MHCII expression on vascular endothelium for the generation of CD4+Foxp3+ Tregs, we next focused on evaluating Tregs in our lung grafts (2). Notably, fewer CD4+ T cells express Foxp3 in B6II− compared with B6 grafts (6.1 ± 0.5 versus 9.4 ± 0.6%; p = 0.009) and the CD8+/CD4+Foxp3+ T cell ratio is significantly altered (Fig. 2G). No such differences were evident in the mediastinal lymph nodes or native right lungs, suggesting a local response (Supplemental Fig. 3A). We did not detect differences in Treg activation markers such as GITR or CTLA4 (Supplemental Fig. 3B), and Tregs isolated from B6 and B6II− lungs were equally potent in inhibiting CD3-stimulated T cell proliferation in vitro (Supplemental Fig. 3C). To examine the role of Tregs in controlling pulmonary inflammation, we treated B6 recipients of B6 lung grafts with PC61. Such treatment resulted in the decrease of CD4+Foxp3+ T cells in the B6 grafts to percentages comparable to those observed in untreated B6II− transplants (5.2 ± 0.8 versus 6.1 ± 0.5 respectively; p = 0.43) (Fig. 2H versus 2G) and was associated with inflammatory changes in B6 grafts similar to those seen in B6II− transplants (Fig. 2H versus 2A). Thus, MHCII expression by lung nonhematopoietic cells is important in regulating the balance between regulatory and effector T cells. It is also conceivable that such inflammatory changes would be more accentuated in grafts derived from mice that lack all four of the classical MHCII genes (15).

We next set out to define a mechanism for the relative deficiency of Tregs in B6II− lungs. We were unable to detect differences in survival, proliferation, or homing patterns of CD4+Foxp3+ Tregs in lungs deficient and sufficient in nonhematopoietic MHCII (Supplemental Fig. 4A–C). To assess whether peripheral generation of Tregs was responsible for the observed differences, we used T cells isolated form mice carrying a GFP-Foxp3 fusion protein-reporter knockin allele (CD4+Foxp3GFP+) (9). We transferred CD4+Foxp3GFP+ cells into B6CD45.1 recipients of B6 or B6II−CD45.1 lungs and found that a significantly higher percentage of graft-infiltrating transferred CD4+Foxp3+ had acquired Foxp3 expression in B6 versus B6II− (Fig. 2I, J). We also observed similar differences in Treg generation between B6 and B6II− grafts in a system of defined nominal Ag presentation (Fig. 2J, bottom panel). Collectively, these data indicate that MHCII expression on pulmonary nonhematopoietic cells plays a role in shaping immune responses through the local generation of Tregs in vivo.

**Tumor growth is attenuated in lungs deficient in nonhematopoietic MHCII**

Despite the differences in inflammatory infiltrate (Fig. 2A), both B6 and B6II− grafts remained ventilated and had identical weights 1 mo (Fig. 3A) or 6 mo posttransplantation (data not shown). When injected with B16 melanoma, significantly more tumor grew in B6 grafts compared with B6II− grafts as evaluated by gross inspection, lung weight, and histology (Fig. 3B). Such differences disappeared if B6 Rag−/− mice were used as recipients, demonstrating that the observed differences in tumor growth were due to alterations in adaptive immune responses (Fig. 3C). We observed a higher number of CD4+ and CD8+ T cells in B6II− grafts compared with B6 grafts, a lower percentage of CD4+ T cells expressing Foxp3 (Fig. 3D), and a higher percentage of melanoma Ag-specific CD8+ T cells (Fig. 3E). Collectively, our data provide evidence that MHCII expression by pulmonary nonhematopoietic cells plays a critical role in the local regulation of tumor growth.

**FIGURE 3.** Tumor immune response. Gross appearance and weights of B6 and B6II− grafts and native right lungs without (A) and after injection of (B) B16 melanoma. H&E staining of grafts indicating melanoma (labeled as tumor) and inflammatory infiltrates (arrows) (original magnification ×100). C, Gross appearance and weights of B6 and B6II− grafts 4 wk after transplantation into B6 Rag−/− mice injected with B16 melanoma. D, Absolute numbers of graft-infiltrating CD4+CD90+ T cells and percentage of graft-infiltrating CD4+ T cells expressing Foxp3 in B6 or B6II− grafts at same time points as shown in B. E, Absolute numbers of CD8+CD90+ T cells and CD8+ T cells with TCR specificity for tyrosinase-related protein 2 in B6 or B6II− grafts at same time points as described in B. Figure summarizes 16 and 19 transplanted mice/group in B6 recipients of B6 and B6II− grafts, respectively, and 7 transplanted mice/group in B6 Rag−/− recipients.
Although it has been shown that peripheral expression of MHCII is important for CD4+ T cell homeostasis, most studies have focused on Ag presentation in lymphoid tissue (16, 17). This is in large part because secondary lymphoid organs are widely considered the predominant, or even exclusive, site for productive interactions between hematopoietic APCs and CD4+ T cells. Unlike hematopoietic APCs that can traffic from nonlymphoid tissue to secondary lymphoid organs, nonhematopoietic cells are a permanent nonmigratory structural component of the lung. Our study now provides compelling in vivo evidence that pulmonary nonhematopoietic cells, through their expression of MHCII, also play a critical role in downregulating CD4+ T cell-mediated immune responses. Our results have implications for the development of therapeutic approaches for multiple pulmonary disease processes.

Disclosures
The authors have no financial conflicts of interest.

References