

Migration and accumulation of eosinophils toward regional lymph nodes after airway allergen challenge

Catherine Duez, PhD,^{a,b} Azzeddine Dakhama, PhD,^a Adrian Tomkinson, PhD,^a Philippe Marquillies, BSc,^b Annette Balhorn, BSc,^a André-Bernard Tonnel, MD,^b Donna L. Bratton, MD,^a and Erwin W. Gelfand, MD^a Denver, Colo, and Lille, France

Background: Eosinophils play a major role in allergic airway inflammation because of their ability to release toxic mediators. In addition, they are able to migrate toward draining thoracic lymph nodes (TLNs) after intratracheal administration, where they can function as antigen-presenting cells.

Objective: In this study, we evaluated *in vivo* eosinophil migration toward the TLN after allergen sensitization and analyzed expression of molecules involved in antigen presentation.

Methods: Mice were sensitized by intraperitoneal injection of ovalbumin on days 1 and 10 and challenged once intranasally with ovalbumin on day 20. The kinetics of eosinophilia was evaluated in blood, lung tissue homogenate, bronchoalveolar lavage fluid, and TLN. Cell surface staining was analyzed by flow cytometry.

Results: The kinetics of eosinophil recruitment was similar in TLN, lung tissue, and blood, beginning at 12 hours and peaking at 48 hours after allergen challenge. Approximately 70% of TLN eosinophils expressed MHC class II molecules, compared with less than 25% in blood and lungs. Moreover, TLN eosinophils expressed higher levels of MHC class II and CD86 compared with blood and lung eosinophils. Most eosinophils expressed CD80 and CD54, whereas only a few eosinophils expressed CD40. Eosinophils in lungs and TLN appeared to be activated with lower CD62-ligand expression compared with blood eosinophils.

Conclusion: The presence of eosinophils with a different phenotype in the TLN at early time points after allergen challenge of sensitized mice supports their capacity to serve as antigen-presenting cells, sustaining allergic/inflammatory responses in the airways. (*J Allergy Clin Immunol* 2004;114:820-5.)

Key words: Eosinophil, airway challenge, allergic inflammation, lymph node, MHC class II

Abbreviations used

BALF: Bronchoalveolar lavage fluid
CD62-L: CD62 ligand
ICAM-1: Intercellular adhesion molecule 1
MFI: Mean fluorescence intensity
TLN: Thoracic lymph node

Eosinophils play an important role in allergic inflammation and helminth infections, in which they function during the acute phase by releasing cytotoxic mediators.¹ However, it has been suggested that eosinophils could also play an important role in the initiation of the immune response as antigen-presenting cells. Human sputum eosinophils from patients with asthma were found to express HLA-DR and ICAM-1, whereas purified blood eosinophils did not.² Culture of blood eosinophils in the presence of GM-CSF, IL-3, or IL-5 induced expression of HLA-DR, ICAM-1, and the costimulatory molecule CD86.^{2,3} HLA-DR-expressing eosinophils mediated antigen-specific proliferation of an autologous HLA-DR-restricted T-cell clone that was inhibitable by anti-HLA-DR and anti-ICAM-1 antibodies.^{2,4} Murine eosinophils were also found capable of processing and presenting antigens to CD4⁺ T cells in the context of MHC class II molecules.⁵

More recently, it has been demonstrated that endobronchial eosinophils from IL-5 transgenic mice were able to capture antigen, migrate to peribronchial lymph nodes, and act as antigen-presenting cells to stimulate CD4⁺ T-cell function.⁶ Moreover, in response to multiple antigen aerosol challenges, eosinophils rapidly migrated toward sites of antigen deposition (airway lumen) and presentation (lung-associated lymph nodes). Eosinophils from bronchoalveolar lavage fluids (BALFs) expressed MHC class II molecules, CD80, and CD86. Antigen-loaded eosinophils purified from peritoneal cavities of IL-5 transgenic mice were able to induce IL-4, IL-5, and IL-13 production by *in vitro* polarized T_H2 cells.⁷ However, unlike dendritic cells, highly purified eosinophils from BALF of ovalbumin-sensitized mice did not induce proliferation of naive T cells in the draining lymph nodes of the lungs.⁸ Not surprisingly, bronchoalveolar lavage eosinophils expressed only weak MHC class II levels. These apparently conflicting reports suggest that

From ^athe Department of Pediatrics, Division of Basic Sciences, National Jewish Medical and Research Center, Denver; and ^bInstitut National de la Santé et de la Recherche Médicale U416, Institut Pasteur de Lille.

Supported by National Institutes of Health grants HL-36577 and HL-61005 to Dr Gelfand and an Institut National de la Santé et de la Recherche Médicale grant to Dr Tonnel. Dr Duez is a recipient of the Vernon Dale Fellowship in Pediatrics at National Jewish Medical and Research Center and a grant from Société de Pathologie Thoracique du Nord/Pas-de-Calais, France.

Received for publication December 23, 2003; revised June 30, 2004; accepted for publication August 9, 2004.

Reprint requests: Erwin W. Gelfand, MD, Department of Pediatrics, National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206. E-mail: gelfande@njc.org.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.08.011

eosinophils may execute different functions in different tissue locations.

The current study was performed to define the kinetics of eosinophil migration and accumulation toward the thoracic lymph nodes (TLNs) in comparison with blood and lung after a single airway allergen challenge. To address regional differences in eosinophil function, we compared eosinophils from all 3 compartments for surface expression of MHC class II (IA antigen), the costimulatory molecules CD40, CD54, CD80, and CD86, and CD62 ligand (CD62-L).

METHODS

Animals

Female BALB/c ByJ mice were obtained from Jackson Laboratories (Bar Harbor, Me). The mice were maintained on ovalbumin-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Sensitization and airway challenge

Groups of mice, 10 to 12 weeks of age, were sensitized by intraperitoneal injection of 20 μ g ovalbumin (Grade V; Sigma Chemical Co, St. Louis, Mo) emulsified in 2.25 mg aluminum hydroxide (AlumImject; Pierce, Rockford, Ill) in a total volume of 100 μ L on days 1 and 10. Mice received a single intranasal challenge with ovalbumin (20 μ L; 5 mg/mL in saline) on day 20. At different time points after ovalbumin challenge, blood, lungs, and TLNs were taken for further analysis. Control, nonsensitized mice received ovalbumin intranasal challenge alone.

Tissue single cell suspension

Blood was taken and added to 1 mL citrate buffer. Platelet depletion was carried out by centrifugation (1000 rpm, 12 minutes), and red blood cells were lysed after 2 rounds of 15-minute incubations in red blood cell lysis buffer (Sigma Chemicals Co). Lungs were perfused with warmed (37°C) PBS containing 0.6 mM EDTA. Lungs were then lavaged via a tracheal tube with phosphate buffered saline solution to obtain the bronchoalveolar lavage (PBS, 1 $\times$ 1 mL). Lung cells were isolated as previously described.⁹ Lungs were removed, cut into pieces, and incubated in 5 mL PBS containing 175 U/mL collagenase (type IA; Sigma Chemical Co) and 10% FCS for 60 minutes at 37°C. The digested lungs were sheared with a sterile 20-gauge needle and filtered through a sterile 45- μ m porous nylon mesh. TLNs were localized as previously described.¹⁰ In non-sensitized but challenged mice, only the 3 parathymic lymph nodes were detectable and dissected, whereas in sensitized and challenged mice, the 3 parathymic lymph nodes as well as the posterior mediastinal lymph node were harvested. Cells were isolated from the lymph nodes by passing the tissue through nylon mesh. Cells were resuspended in PBS and counted with a hemocytometer.

Staining and flow cytometry analysis

Differential cell counts were performed on bronchoalveolar lavages by counting at least 200 cells on cytocentrifuged preparations, stained with May Grünwald Giemsa (Labonord, Lille, France), and differentiated by standard hematologic procedures.

Cells isolated from blood, lung, and TLN were resuspended at a concentration of 5 $\times$ 10⁶ cells/mL in PBS. Cells (0.5 $\times$ 10⁶) were stained for 30 minutes on ice with an antibody cocktail containing fluorescein isothiocyanate-conjugated anti-CCR3 (R&D Systems, Abingdon, United Kingdom), allophycocyanin-conjugated anti-CD3

(PharMingen, Becton Dickinson, San Diego, Calif), and 1 of the following activation markers: phycoerythrin-conjugated anti-IA, anti-CD40, anti-CD54, anti-CD-80, anti-CD86, or anti-CD62-L (PharMingen, Becton Dickinson), or their corresponding isotype-matched control antibodies. After washing, stained cells were analyzed by flow cytometry (FACSCALIBUR, Becton Dickinson). Spleen cells from IL-5 transgenic mice¹¹ were used to locate mouse eosinophils on the forward and side scatter window. Eosinophils located in this gate were identified by a positive CCR3-staining and negative CD3-staining, as previously shown and characterized by standard hematologic staining after sorting of this cell population by flow cytometry.⁸ This analysis was then used to evaluate the percentage of positive cells and the mean fluorescence intensity (MFI) obtained with phycoerythrin-conjugated antibodies. The results are expressed as percentage of positive cells and MFI after deduction of the values obtained with isotype control antibody.

Statistical analysis

Data (n = 6 mice per group) are expressed as means $\pm$ standard errors (SEMs). Statistical significance was determined by ANOVA by using Statview 4.5 statistical analysis software package (Abacus Concepts, Berkeley, Calif). Differences between the groups were determined by multiple comparisons of the means by using the Fisher protected least significant difference test.

RESULTS

Kinetics of tissue eosinophil infiltration after single intranasal ovalbumin challenge

As shown in Table I, the numbers of circulating eosinophils were significantly increased in the blood of sensitized mice at 12 hours and 48 hours after intranasal ovalbumin challenge ($P < .01$). To appreciate trafficking patterns to the lung tissue more closely, we detected eosinophils in lung tissue homogenates and found a similar kinetics as in the blood, with a peak at 48 hours. In the BALF, the increase in eosinophil numbers was delayed and began at 48 hours. Interestingly, a low but significant number of eosinophils were detected in TLNs beginning at 12 hours, with a further increases at 48 hours after allergen challenge; baseline levels were regained at 96 hours (data not shown). Thus, airway challenge in sensitized mice induces an increase in numbers of eosinophils in the bloodstream, as well as migration to the airway tissue and local draining lymph nodes.

Cell surface marker expression on eosinophils in the different tissue compartments

In an attempt to delineate potential functions of eosinophils located in the TLNs, we compared cell surface expression of MHC class II: IA and other molecules involved in antigen presentation to expression on blood and lung eosinophils at 48 hours, corresponding with the peak of migration to TLN.

The numbers of TLN eosinophils (approximately 70%) that expressed MHC class II molecules were significantly higher compared with eosinophils in blood and lungs (<25%). Further, the levels of expression of MHC class II were higher compared with eosinophils found in blood and lungs (geometric MFI, 235 $\pm$ 120 on TLN

TABLE I. Kinetics of allergen-induced eosinophil recruitment and accumulation in blood, lung, and TLNs in sensitized mice

Tissue compartment	Naive control group		Sensitized group	
	Time postchallenge, 48 h			
Peripheral blood*	7500 (2236)	15,450 (2051)	22,100‡ (1869)	107,250‡§ (7610)
Lung tissue*	7225 (1656)	9617 (453)	10,467 (1342)	29,325‡§ (7185)
Bronchoalveolar lavage†	0 (0)	0 (0)	0 (0)	10,520‡§ (3620)
TLNs*	355 (203)	178 (28)	1500‡ (508)	2047‡ (259)

*Values are means and (SEMs) eosinophil counts per 10^6 total cells recovered from blood (excluding red blood cells), whole lung tissue digest, and TLNs.

†(2): Values are means and (SEMs) total eosinophils recovered in the BALF.

‡Significant difference compared with 0-hour time point; $P < .01$.

§Significant difference compared with 12-hour time point; $P < .01$.

||Significant difference compared with naive control group; $P < 0.01$.

eosinophils, compared with 24 ± 10 and 110 ± 59 on blood and lung eosinophils, respectively; Fig 1). Analysis of costimulatory molecule expression showed that all tissues had similar numbers of eosinophils expressing CD86 (approximately 13%), but that TLN eosinophils expressed significantly higher levels of CD86 than blood or lung eosinophils (MFI, 94 ± 17 , 17 ± 10 , 16 ± 6 , respectively; Fig 2). Similarly, CD80 was detected on more than 70% of eosinophils from each of the tissues, but a significantly higher level of expression was detected in TLN eosinophils compared with eosinophils obtained from blood and lungs (MFI, 78 ± 7 , compared with 37 ± 12 and 59 ± 5 in blood and lung, respectively; Fig 3). Only a few eosinophils expressed CD40, but positive cells in the TLN expressed the highest levels (Fig 4). Most eosinophils expressed CD54 in all tissues (Fig 5). Importantly, eosinophils in lungs and TLN demonstrated evidence for activation,¹² because the numbers expressing CD62-L were low; $93\% \pm 3\%$ of blood eosinophils expressed CD62-L, compared with $3\% \pm 2\%$ and $7\% \pm 1\%$ in lungs and TLN, respectively (Fig 6).

DISCUSSION

In this study, we demonstrated that eosinophils migrate and accumulate in the draining lymph nodes of the lungs (TLNs) after a single intranasal antigen challenge in sensitized (but not in nonsensitized) mice. Eosinophil migration was detected at 12 hours and peaked at 48 hours, whereas the numbers returned to baseline levels at 96 hours. The kinetics of eosinophil recruitment were quite similar in lung tissue and blood, although peribronchial eosinophilia persisted at 96 hours. Lung tissue eosinophilia as reported here paralleled changes in lung resistance: airway hyperresponsiveness was observed in sensitized and challenged mice at 24 and 48 hours after challenge, but resolved by 96 hours.¹³ These results and others define the sequential eosinophilia, which develops in peripheral blood and lungs after airway challenge of

sensitized mice and suggests an eosinophil migration pathway from bone marrow to blood to lungs.^{14,15} Although our results (similar kinetics of eosinophil recruitment in lymph node and lungs) might suggest the recruitment of blood eosinophils into TLNs, other results have argued that eosinophils migrate from the lungs to the TLNs; airway eosinophils injected intratracheally were detected 8 hours later in the TLNs,⁶ whereas airway eosinophils injected intravenously were not.⁷ Our data showing an early recruitment of eosinophils in TLNs without a parallel increase in the number of these cells in the lung at 12 hours, but a concomitant increase in eosinophil numbers in all tissue compartments at 48 hours, may suggest a 2-phase recruitment for these cells into TLN: an early phase from peripheral blood followed by a late phase involving recruitment from lung tissue. Whether distinct chemokines and chemokine receptors are involved in both phases of recruitment needs to be defined in future studies. Eotaxin plays an important role in allergen-induced eosinophil accumulation in the lung,¹⁶ but it does not appear to be required for trafficking of eosinophils from the airway lumen to the lymph nodes during allergen challenge, because both $CCR3^{-/-}$ and $CCR3^{+/+}$ eosinophils migrate equally in this direction.⁶ This suggests that other chemokines/chemokine receptors are involved in differential regulation of eosinophil mobilization from 1 tissue compartment to another.

The particular localization of eosinophils in the TLN and the phenotypic alterations demonstrated here after airway challenge is in keeping with the concept that these eosinophils may participate in antigen presentation and T-lymphocyte activation. Eosinophils isolated from ovalbumin-sensitized mice expressed different molecules involved in T-cell activation, both constitutively and inducibly. Indeed, in the current study, eosinophils isolated from the TLN (and lungs) appeared activated, as suggested by the decreased expression of CD62-L, as previously shown for BALF, but not blood eosinophils.¹² Although eosinophils constitutively expressed CD80 and CD54 in all of these compartments (blood, lungs, and

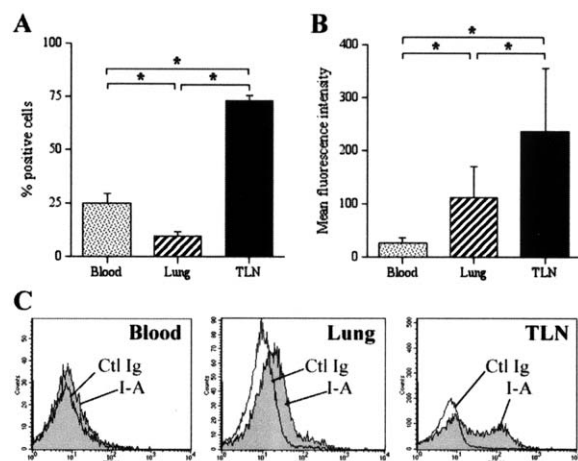


FIG 1. Expression of MHC class II on eosinophils from blood, lung, and TLNs 48 hours after ovalbumin challenge. (A) Percentage of MHC class II (I-A)-positive eosinophils, and (B) geometric MFI, obtained after subtraction of background values obtained by staining with isotype-matched control antibodies. *Significant difference ($P < .01$) between the different tissue compartments. (C) Representative histograms of MHC class II expression on eosinophils from blood, lungs, and TLNs.

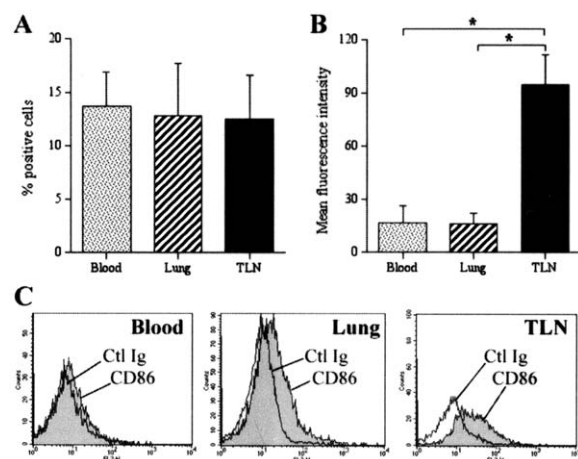


FIG 2. Expression of CD86 on eosinophils from blood, lung, and TLNs 48 hours after ovalbumin challenge. (A) Percentage of CD86-positive eosinophils and (B) geometric MFI, obtained after subtraction of background values obtained by staining with isotype-matched control antibodies. *Significant difference ($P < .01$) between the different tissue compartments. (C) Representative histograms of CD86 expression on eosinophils from blood, lung, and TLNs.

TLN), only TLN and lung expressed MHC class II molecules, whereas CD86 was induced in TLN eosinophils only.

Expression of MHC class II and costimulatory molecules involved in antigen presentation and T-cell activation has been shown on human and mouse eosinophils. In human beings, ICAM-1 and HLA-DR are expressed on sputum eosinophils but not on blood eosinophils.¹⁷ Synergy between IL-3, IL-5, or GM-CSF and TNF- α was found mainly responsible for ICAM-1 induction, whereas

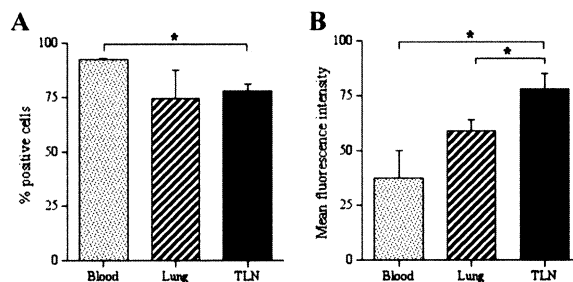


FIG 3. Expression of CD80 on eosinophils from blood, lung, and TLNs 48 hours after ovalbumin challenge. (A) Percentage of CD80-positive eosinophils and (B) geometric MFI, obtained after subtraction of background values obtained by staining with isotype-matched control antibodies. *Significant difference ($P < .01$) between the different tissue compartments.

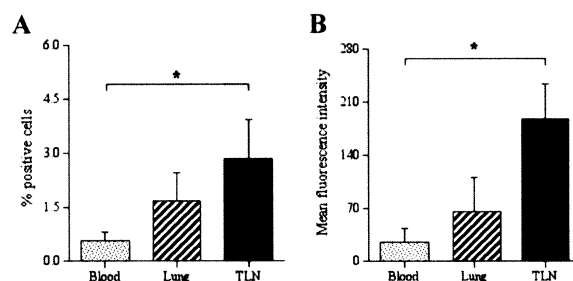


FIG 4. Expression of CD40 on eosinophils from blood, lung, and TLNs 48 hours after ovalbumin challenge. (A) Percentage of CD40-positive eosinophils and (B) geometric MFI, obtained after subtraction of background values obtained by staining with isotype-matched control antibodies. *Significant difference ($P < .01$) between the different tissue compartments.

synergy between IL-3, IL-5, or GM-CSF and IFN- γ was shown for HLA-DR expression. ICAM-1-bearing eosinophils have increased adhesion capacity for autologous T cells, whereas HLA-DR-expressing eosinophils mediated antigen-specific T-cell proliferation that was inhibitable by anti-HLA-DR and anti-ICAM-1 antibodies.² IL-3-treated human eosinophils express HLA-DR and CD86 but not CD80 and support T-cell proliferation in response to a superantigen as well as proliferation of specific T-cell clones.³ GM-CSF and IL-5-treated eosinophils can present native antigen to T cells; nonetheless, the magnitude of T-cell proliferation to antigen in the presence of eosinophils is very modest compared with that in the presence of monocytes.^{4,5,18,19} All of these cytokines (GM-CSF, IL-3, IL-5, TNF- α and IFN- γ) can be found in the lung environment of sensitized and challenged mice, as well as in the draining lymph nodes where T cells are activated. This could explain the increased expression of costimulatory molecules on eosinophils found in lung and TLN compared with blood.^{13,20}

The expression of CD80 and CD86 on airway eosinophils reported in the current study was similar to that described by Shi et al,⁶ with a higher expression of CD80. After intratracheal administration, these eosinophils enter paratracheal lymph nodes within 8 hours, peaking at 24

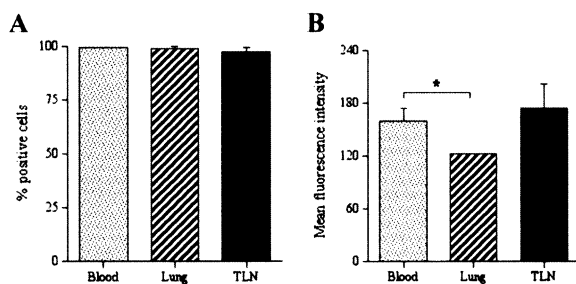


FIG 5. Expression of CD54 on eosinophils from blood, lung, and TLNs 48 hours after ovalbumin challenge. **(A)** Percentage of CD54-positive eosinophils and **(B)** geometric MFI, obtained after subtraction of background values obtained by staining with isotype-matched control antibodies. *Significant difference ($P < .01$) between the different tissue compartments.

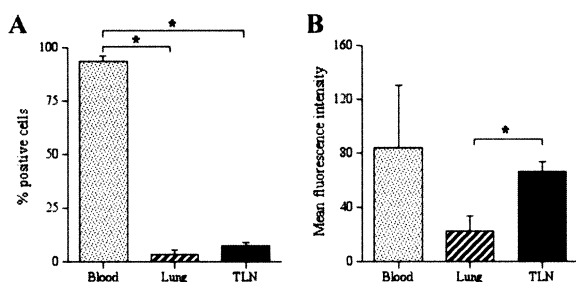


FIG 6. Expression of CD62-L on eosinophils from blood, lung, and TLNs 48 hours after ovalbumin challenge. **(A)** Percentage of CD62-L-positive eosinophils and **(B)** geometric MFI after subtraction of background values obtained by staining with isotype-matched control antibodies. *Significant difference ($P < .01$) between the different tissue compartments.

hours and persisting for at least 120 hours. When instilled to the airways, approximately 10% of eosinophils became resident at 24 hours within the sampled paratracheal lymph nodes. By using a different airway challenge protocol, MacKenzie et al⁷ showed that eosinophils migrate toward sites of antigen deposition (airway lumen) and presentation (lung-associated lymph nodes) after multiple antigen aerosol challenge of sensitized mice. In this study, eosinophils purified from BALFs also expressed MHC class II, and antigen-loaded eosinophils purified from peritoneal cavities of IL-5 transgenic mice were able to induce IL-4, IL-5, and IL-13 production from *in vitro* polarized T_H2 cells.⁷

A recent study showed that even though airway eosinophils accumulate in the TLN, they lack the capacity for antigen presentation to naive T cells.⁸ However, the eosinophils used in T-cell cocultures were isolated from BALFs and not from the TLN. These eosinophils exhibited low-level expression of MHC class II molecules, different from what we demonstrated with a higher number of TLN eosinophils expressing MHC class II than in blood and lungs. Further, the TLN eosinophils showed the highest level of MHC class II expression; lung eosinophils had an intermediate expression, between

blood and TLN eosinophils. The reasons for increased levels of MHC class II and CD86 expression in TLN are not known, but they could be the result of persistent allergen exposure, as shown for dendritic cells.²¹ Taken together, the results indicate that TLN eosinophils express a large variety of surface molecules that can facilitate interactions with T cells, and identify the potential role for TLN eosinophils in costimulation of naive and/or memory T cells.

The authors thank L. N. Cunningham and D. Nabighian (National Jewish Medical and Research Center) for their assistance.

REFERENCES

- Dombrowicz D, Capron M. Eosinophils, allergy and parasites. *Curr Opin Immunol* 2001;13:716-20.
- Hansel TT, De Vries IJ, Carballido JM, Braun RK, Carballido-Perrig N, Rihs S, et al. Induction and function of eosinophil intercellular adhesion molecule-1 and HLA-DR. *J Immunol* 1992;149:2130-6.
- Celestin J, Rotschke O, Falk K, Ramesh N, Jabara H, Strominger J, et al. IL-3 induces B7.2 (CD86) expression and costimulatory activity in human eosinophils. *J Immunol* 2001;167:6097-104.
- Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils: HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. *J Immunol* 1993;150:2554-62.
- Del Pozo V, De Andres B, Martin E, Cardaba B, Fernandez JC, Gallardo S, et al. Eosinophils as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. *Eur J Immunol* 1992;22:1919-25.
- Shi H-Z, Humbles A, Gerard C, Jin Z, Weller PF. Lymph node trafficking and antigen presentation by endobronchial eosinophils. *J Clin Invest* 2000;105:945-53.
- MacKenzie JR, Mattes J, Dent LA, Foster PS. Eosinophils promote allergic disease of the lung by regulating CD4+ Th2 lymphocyte function. *J Immunol* 2001;167:3146-55.
- van Rijst LS, Vos N, Hijdra D, de Vries VC, Henk C, Hoogsteden HC, Lambrecht BN. Airway eosinophils accumulate in the mediastinal lymph nodes but lack antigen-presenting potential for naive T cells. *J Immunol* 2003;171:3372-8.
- Oshiba A, Hamelmann E, Takeda K, Bradley KL, Loader JE, Larsen GL, et al. Passive transfer of immediate hypersensitivity and airway hyper-responsiveness by allergen-specific immunoglobulin (Ig) E and IgG1 in mice. *J Clin Invest* 1996;97:1398-408.
- Tilney NL. Patterns of lymphatic drainage in the adult laboratory rat. *J Anat* 1971;109:369-83.
- Dent LA, Strath M, Mellor AL, Sanderson CJ. Eosinophilia in transgenic mice expressing interleukin 5. *J Exp Med* 1990;172:1425-31.
- Shinagawa K, Anderson G. Rapid isolation of homogeneous murine bronchoalveolar lavage fluid eosinophils by differential lectin affinity interaction and negative selection. *J Immunol Methods* 2000;237:65-72.
- Tomkinson A, Cieslewicz G, Duez C, Larson KA, Lee JJ, Gelfand EW. Temporal association between airway hyperresponsiveness and airway eosinophilia in ovalbumin-sensitized mice. *Am J Respir Crit Care Med* 2001;163:721-30.
- Kung TT, Stelts D, Zurcher JA, Watnick AS, Jones H, Mauser PJ, et al. Mechanisms of allergic pulmonary eosinophilia in the mouse. *J Allergy Clin Immunol* 1994;94:1217-24.
- Hogan MB, Weissman DN, Hubbs AF, Gibson LF, Piktet D, Landreth KS. Regulation of eosinophilopoiesis in a murine model of asthma. *J Immunol* 2003;171:2644-51.
- Humbles AA, Lu B, Friend DS, Okinaka S, Lora J, Al-Garawi A, et al. The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proc Natl Acad Sci U S A* 2002;99:1479-84.
- Hansel TT, Braunstein JB, Walker C, Blaser K, Bruijnzeel PL, Virchow JC, et al. Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. *Clin Exp Immunol* 1991;86:271-7.

18. Mawhorter SD, Kazura JW, Boom WH. Human eosinophils as antigen-presenting cells: relative efficiency for superantigen- and antigen-induced CD4+ T-cell proliferation. *Immunology* 1994;81:584-91.
19. Tamura N, Ishii N, Nakazawa M, Nagoya M, Yoshinari M, Amano T, et al. Requirement of CD80 and CD86 molecules for antigen presentation by eosinophils. *Scand J Immunol* 1996;44:229-38.
20. Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;111:450-63.
21. Hammad H, Charbonnier A-S, Duez C, Jacquet A, Stewart GA, Tonnel A-B, et al. Th2 polarization by Der p 1-pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood* 2001;98:1135-41.