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Cutting Edge

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Cutting Edge: CD49d$^+$ Neutrophils Induce FcεRI Expression on Lung Dendritic Cells in a Mouse Model of Postviral Asthma

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The increasing prevalence of atopy and asthma remains unexplained but may be due to infection with respiratory viruses. In support of this hypothesis, we showed that experimental asthma after viral infection in mice depended on type I IFN-driven upregulation of FcεRI on conventional dendritic cells (cDCs) in the lung. In this article, we demonstrate that FcεRI expression on lung cDCs depends on an unexpected activity of a CD49d$^+$ subset of polymorphonuclear neutrophils (PMNs) that are found in the lungs of wild-type C57BL6 but not mice deficient in type I IFNR. Expression of FcεRI depends in part on a CD11b-dependent interaction between PMNs and cDCs. This study demonstrates a PMN–cDC interaction in the lung that is necessary for the ability of viral infection to induce atopic disease. ***The Journal of Immunology*****, 2010, 185: 4983–4987.**

The increasing prevalence of asthma and atopic disease is a major public health problem (1). Many hypotheses have been proposed to explain this epidemic of allergic disease (2, 3). One hypothesis is based on epidemiological studies that have correlated severe viral infections early in life to the subsequent development of asthma and allergic disease (4–6). However, until recently, mechanistic studies were lacking to indicate how viral infection could lead to atopic disease.

Recently, using a mouse model of asthma triggered by a transient viral infection, we identified a potential mechanism by which a Th1 antiviral response induces Th2 atopic disease. We demonstrated that mice that survive a severe Sendai virus (SeV) infection develop chronic airway hyperresponsiveness and mucous cell metaplasia, similar to human infants infected with respiratory syncytial virus (7). We also showed that acute development of airway hyperresponsiveness was dependent on the expression of FcεRI on conventional dendritic cells (cDCs) in the lung. The increased cDC FcεRI expression in the lung during SeV infection required intact type I IFNR signaling. Importantly, cross-linking of FcεRI led to production of CCL28 and recruitment of IL-13–producing Th2 cells, which in turn drove the subsequent development of chronic asthma (8). Therefore, blocking induction of FcεRI on the cDC has clear therapeutic implications in preventing postviral atopic disease.

The present study sought to extend our prior observations and identify the specific cells involved in the type I IFN-dependent induction of FcεRI on lung cDCs following SeV infection.

Materials and Methods

Mouse generation and handling

C57BL6 mice were from The Jackson Laboratory (Bar Harbor, ME). Mice deficient in type I IFNR (*IFNAR*$^{-/-}$; C57BL6 background) were a kind gift of J. Sprent (Garvan Institute, Sydney, Australia). Mice 6–20 wk old were used for all experiments. Mice were housed, handled, and experiments performed according to protocols approved by the Institutional Animal Care and Use Committee at the Medical College of Wisconsin (Milwaukee, WI).

Mice were inoculated intranasally (i.n.) with 2×10^5 PFU SeV (Fushimi strain; American Type Culture Collection, Manassas, VA) and monitored daily for weight and activity. A total of 100 μg anti–Gr-1, anti-NK1.1, anti-murine plasmacytoid DC Ag-1 (mPDCA-1), or control IgG mAb were given s.c. on days −1, 1, 3, and 5 postinoculation (PI) SeV. In some experiments, mice were inoculated i.n. with 10 μg LPS (from *Escherichia coli* O55:B5, Sigma-Aldrich, St. Louis, MO) and sacrificed 1 d PI.

Cell purification and culture

Lung cDCs were obtained from lung digest as previously described (9). Briefly, mice were euthanized, the inferior vena cava severed, and the right cardiac ventricle injected with PBS, before bronchoalveolar lavage (BAL) was performed with 1 ml PBS. Lungs were removed, minced, and incubated in digest media for 1 h at 37°C, and EDTA was added to the media (2 mM final concentration) for the last 15 min. The single-cell suspension was filtered through 40-μm pore cell strainers before removing erythrocytes by NH_4Cl hypotonic lysis (Sigma-Aldrich). cDCs were purified using positive immunomagnetic selection with CD11c MACS beads (Miltenyi Biotec, Auburn, CA), with >95% purity achieved after two serial purifications (8).

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Abbreviations used in this paper: BAL, bronchoalveolar lavage; cDC, conventional dendritic cell; CEACAM-1, carcinoembryonic Ag cell adhesion molecule-1; DC-SIGN, dendritic cell-specific ICAM-3–grabbing nonintegrin; IFNAR$^{-/-}$, type I IFNR deficient; i.n., intranasally; MFI, mean fluorescence intensity; mPDCA-1, murine plasmacytoid dendritic cell Ag-1; PI, postinoculation; PMN, polymorphonuclear neutrophil; SeV, Sendai virus; WT, wild-type.

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Polymorphonuclear neutrophils (PMNs; ≥85% pure) were isolated from BAL of mice 1 or 3 d PI. Subsets of PMNs were obtained by sorting for CD49d (FACSAria, BD Biosciences, San Jose, CA). PMNs from BAL of SeV-infected mice and lung cDCs from uninfected mice were cultured together for 48 h in complete RPMI 1640 (Sigma-Aldrich) supplemented with 10% FCS and penicillin/streptomycin (Invitrogen, Carlsbad, CA) media at 37°C with 5% CO_2. PMN viability was ≥95% at the start of culture and decreased to 38 ± 2.6% by 48 h.

For IFN treatment of PMNs, 1000 U/ml mouse IFN-β (R&D Systems, Minneapolis, MN) was added to PMNs from BAL of mice 1 d PI LPS or to PMNs purified from naive bone marrow and cocultured with cDCs.

Purification of PMNs from bone marrow

PMNs were isolated from bone marrow as previously described (10). Marrow was flushed from long bones with HBSS/0.1% BSA, pelleted, and resuspended in 3 ml 45% Percoll (GE Healthcare Biosciences, Piscataway, NJ). Solutions of 66, 60, 55, and 50% were prepared by diluting the 100% stock Percoll with HBSS. A total of 3 ml 66% solution and 2 ml aliquots of each decreasing concentration of Percoll solution were layered over one another in a 15-ml conical tube. The bone marrow single-cell suspension in 45% Percoll was subsequently layered over the prepared Percoll density gradient, followed by centrifugation at 1800 × *g* for 30 min at room temperature. Cells were collected from the 66–60% interface and washed with HBSS/0.1%BSA. PMN purity was consistently >95% as assessed by flow cytometry. Contaminating cells found at this interface included a small percentage of nucleated erythrocytes and B cells.

Abs and flow cytometry analyses

Phytoerythrin-, allophycocyanin-, FITC-, or Alexa Fluor 647-labeled Abs against mouse CD11c (clone N418), FcεRIα (clone MAR-1), CD49d (clone R1-2), Gr-1 (clone RB6-8C5), and isotype control IgGs were obtained from eBioscience (San Diego, CA), BD Pharmingen (San Diego, CA), and/or Biolegend (San Diego, CA). Anti–Gr-1 (clone RB6-8C5), anti-NK1.1 (clone PK136), anti–mPDCA-1 (clone eBio927), anti-CD11b (clone M1/70), and anti-carcinoembryonic Ag cell adhesion molecule-1 (anti–CEA-CAM-1; clone CC1) mAbs were obtained from eBioscience or BD Pharmingen. Stained cells were analyzed with a FACSCalibur flow cytometer (BD Biosciences) and data analyzed with FlowJo software (Tree Star, Ashland, OR).

Morphologic examination

Cells were mounted onto slides using the Shandon Cytospin 4 (Thermo Fisher Scientific, Waltham, MA) at 300 rpm for 5 min, and slides were stained with the Diff-Quick Stain Kit as per manufacturer's instructions (Thermo Fisher Scientific).

Immunohistochemistry

Whole lung from SeV-infected mice frozen in Tissue-Tek O.C.T. compound (Sakura, Hayward, CA), and 10-μm sections were obtained with a cryostat. Sections were fixed with acetone, blocked with goat serum, and stained with FITC-labeled anti–VCAM-1 (clone 429) mAb or IgG isotype control (Biolegend). Stained lung sections were then examined for fluorescence by a blinded observer.

Real-time PCR assay

mRNA was isolated from whole lung using TRIzol (Sigma-Aldrich). cDNA was then generated with the QuantiTect reverse transcription kit (Qiagen, Valencia, CA) per the manufacturer's instructions. Quantitative PCR assays were performed using StepOnePlus PCR system (Applied Biosystems, Foster City, CA) and TaqMan Fast Universal PCR Master Mix. TaqMan primer and probes for rodent GAPDH control (4352339E), VCAM-1 (Mm01320970_m1), and TNF-α (Mm00443258_m1) were obtained from Applied Biosystems.

Statistical analyses

Unless otherwise stated, all data are presented as mean ± SEM. Student *t* test was used to assess statistical significance between means. Mann-Whitney *U* test was used for comparison of medians of nonparametric data. For comparison of ratios, Wilcoxin signed rank was used. In all cases, significance was set at $p < 0.05$.

Results and Discussion

PMNs are required for SeV-mediated FcεRI expression on cDCs

We reported previously that lung cDCs express FcεRI by SeV PI day 3, which suggested that an early effector cell was involved.

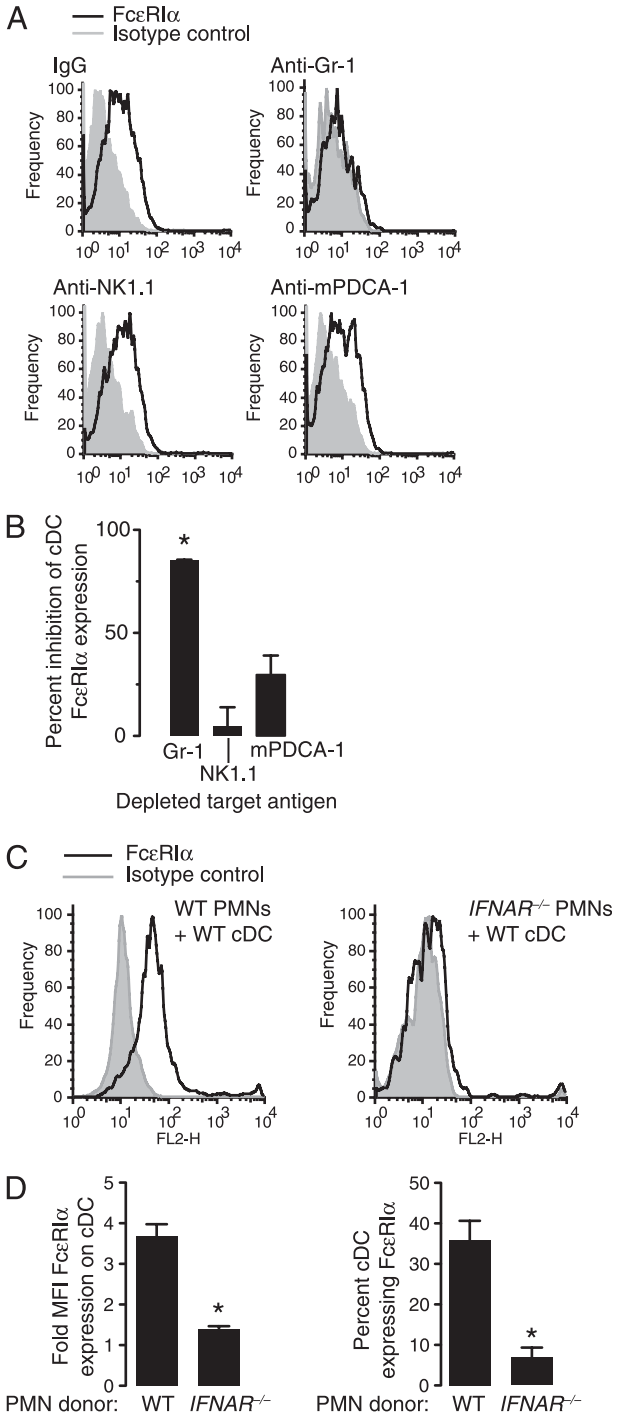

FIGURE 1. PMNs from IFNAR-sufficient mice drive expression of FcεRI on lung cDCs. *A*, Representative histograms of FcεRIα expression on purified lung cDCs 5 d PI with SeV in mice given 100 μg control IgG, anti–Gr-1, anti-NK1.1, or anti–mPDCA-1 mAb s.c. on days −1, 1, 3, and 5 PI. *B*, Only anti–Gr-1 treatment significantly inhibited expression of FcεRIα on lung cDCs. Data from three mice per group in two separate experiments (total of $n = 6$ mice/group). $^*p < 0.05$ versus control IgG group. *C*, Representative histograms of FcεRIα expression on purified lung cDCs after 48 h culture with PMNs from the BAL of day 3 PI SeV WT or *IFNAR*$^{-/-}$ mice. *D*, MFI of FcεRIα on cDC cultured as in *C* and percent of cDCs expressing FcεRIα postculture as in *C*. Data from a total of 12 BAL donor and 4 cDC donor mice per group used for two separate experiments ($n = 6$ BAL and 2 cDC donors/experiment). $^*p < 0.05$ versus WT.

PMNs are important effector cells in SeV induced lung disease and constitute >90% of the cells in the BAL of SeV-infected mice at day 3 PI (11, 12). Therefore, we depleted PMNs and examined FcεRI expression on cDCs (11, 12). Treating C57BL6 mice with anti–Gr-1 mAb every other day from 1 d prior to SeV inoculation blocked the upregulation of FcεRI expression on cDC (Fig. 1*A*, 1*B*). Because anti–Gr-1 mAb has been reported to cross-react with Ly-6C–expressing cells, we examined cDC FcεRI expression when mice were treated with depleting mAbs against NK cells and plasmacytoid DCs, the two major Ly-6C–bearing cell types present during the early time points of the infection (13–15). NK cell or plasmacytoid DC-depleting mAbs anti-NK1.1 or anti–mPDCA-1, respectively, failed to significantly reduce FcεRI expression on cDCs (Fig. 1*A*, 1*B*). These findings suggested that PMNs were required for SeV-mediated induction of FcεRI on lung cDCs.

Next, we tested whether PMNs could induce FcεRI on the cDC in vitro. By performing transwell studies, we found that purified naive lung cDCs were induced to express FcεRI after 48 h of culture with PMNs isolated from the BAL of day 3 PI SeV infected wild-type (WT) mice. This effect was seen only when cDCs were cultured in direct contact with PMNs and did not occur when the cells were cultured for only 24 h (data not shown). Moreover, consistent with previous reports on cross talk between PMNs and DCs, a 10:1 excess of PMNs provided the strongest signal for cDC FcεRIα induction (data not shown) (16).

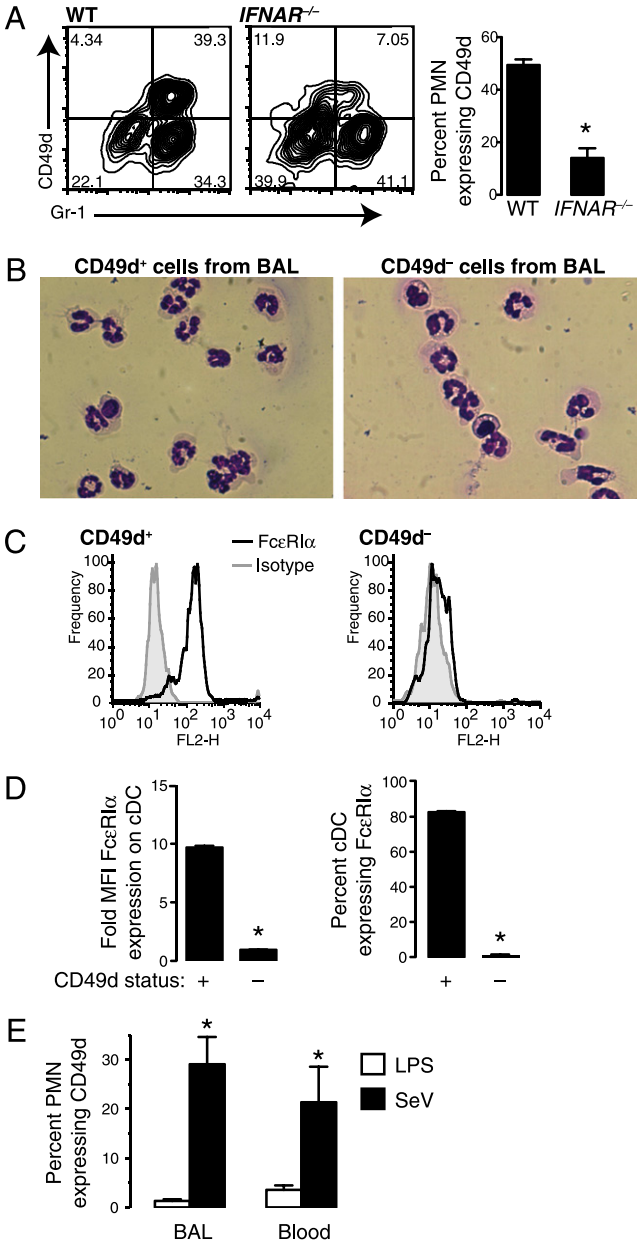

FIGURE 2. SeV infection leads to accumulation of CD49d$^+$ PMNs, which induce FcεRIα on lung cDCs. *A*, Representative contour plots for Gr-1 versus CD49d expression on BAL cells from day 3 PI SeV-infected WT and *IFNAR*$^{-/-}$ mice and quantification of these data (mean ± SEM; $n = 5$ per group). *B*, CD49d$^+$ and CD49d$^-$ PMNs have similar morphology. *C*, CD49d$^+$ PMNs are sufficient to drive FcεRI expression on cDCs. Representative histograms showing expression of FcεRIα on lung cDCs after 48 h culture with CD49d$^+$ PMNs or CD49d$^-$ PMNs from BAL of day 3 PI SeV-infected WT mice. *D*, Quantification of data in *C*, showing mean ± SEM fold MFI and percent of cDCs expressing FcεRI postculture with CD49d$^+$ and CD49d$^-$ PMNs. Data from a total of 20 BAL donor mice and 4 cDC donor mice split into two separate experiments (10 BAL and 2 cDC donors/experiment). $^*p <$ 0.05 versus CD49d$^+$. *E*, SeV but not LPS inoculation specifically induces the CD49d$^+$ PMNs. WT mice were treated with LPS or SeV i.n. and 1 d later sacrificed and BAL and blood removed. Expression of CD49d versus Gr-1 was determined by flow cytometry, with mean ± SEM percent of PMNs in the BAL and blood expressing CD49d shown ($n = 4$ per group). $^*p < 0.05$.

PMNs from IFNAR$^{-/-}$ *mice fail to induce FcεRI expression on cDCs*

We previously demonstrated that type I IFN acted on a cell type other than the cDC to induce FcεRI expression on lung cDC during SeV infection (8). Therefore, we next determined if type I IFN signaling was necessary for the PMNs to induce FcεRI on cDCs. PMNs were isolated from the BAL of day 3 PI SeV-infected WT or *IFNAR*$^{-/-}$ mice and cocultured with lung cDCs purified from naive WT mice. In contrast to PMNs isolated from *IFNAR*$^{-/-}$ mice, PMNs from WT mice

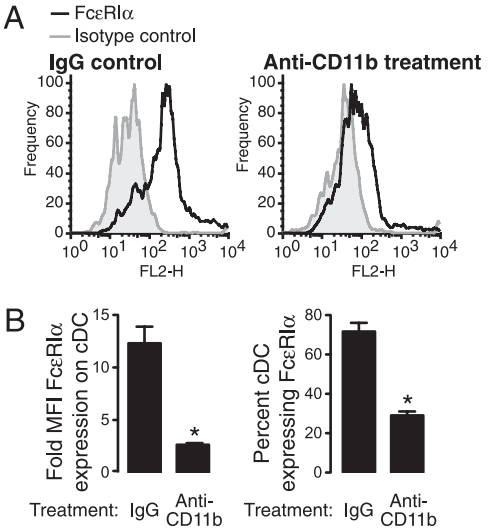

FIGURE 3. PMNs induce FcεRI on cDCs through a partial CD11b dependent process. *A*, CD49d$^+$ PMNs sorted from BAL of day 3 PI SeV-infected WT mice were treated with an IgG control mAb or a blocking anti-CD11b mAb prior to culture with lung cDCs. Representative histograms showing FcεRIα expression on lung cDCs after 48 h culture. *B*, Quantification of data in *A* showing mean ± SEM fold MFI and percent of cDCs expressing FcεRI postculture. Data from a total of 20 BAL donor and 4 cDC donor mice split into two separate experiments (10 BAL and 2 cDC donors/experiment). $^*p < 0.05$.

induced the expression FcεRI on lung cDCs after 48 h (Fig. 1*C*, 1*D*).

CD49d expression defines a subset of PMNs required for FcεRIα induction

PMNs have been grouped into two distinct functional subsets based in part on the surface expression of CD49d (17). In contrast to PMNs isolated from WT mice, very few PMNs isolated from the BAL on day 3 PI from *IFNAR*$^{-/-}$ mice expressed CD49d (Fig. 2*A*). This result suggested that the CD49d-expressing subset of PMNs might be important for induction of FcεRI on lung cDCs. Therefore, PMNs from WT BAL were purified by cell sorting into the CD49d^{+} or CD49^{-} populations. As shown in Fig. 2*B*, both of these populations appeared morphologically similar with typical features of PMNs. These subsets of PMNs were separately cultured with naive cDCs for 48 h. Only CD49d^{+} PMNs were capable of inducing FcεRIα expression on lung cDCs (Fig. 2*C*, 2*D*).

Because we had previously shown a type I IFN dependence in this response, we wanted to determine if type I IFN induced expression of CD49d on PMNs. Bone marrow-derived PMNs were cultured with murine IFN-β; however, CD49d expression was not induced (data not shown). Further, coculturing naive WT bone marrow PMNs with naive lung cDCs in the presence of IFN-β did not result in expression of FcεRI on the cDC (data not shown). Therefore, it appears that the effect of type I IFN in this response is indirect, acting on a cell other than the PMNs.

Because CD49d is an adhesion molecule that binds VCAM, we explored the possibility that differential expression of VCAM-1 or TNF (which induces VCAM-1 expression) in the lungs of WT and *IFNAR*$^{-/-}$ mice with SeV infection could explain the difference in accumulation of CD49d^{+} PMNs (18). However, using histology, we did not find any difference in VCAM-1 protein on the lung endothelium of WT or *IFNAR*$^{-/-}$ mice. In addition, we did not find any difference in whole lung *TNF* or *VCAM-1* message (data not shown). Thus, differential VCAM-1 expression is not the mechanism through which CD49d^{+} PMNs accumulate in the lungs of WT mice.

Viral specificity of the response

LPS treatment of WT mice failed to induce FcεRI expression on lung cDCs (data not shown). Therefore, we compared the frequency of CD49d^{+} PMNs in the BAL or blood of mice following LPS treatment or infection with SeV. Because LPS causes a rapid influx of PMNs in the first 24 h post-administration, we examined the PMNs isolated from the BAL or blood 1 d following administration of LPS or SeV. CD49d^{+} PMNs were found only in the BAL or blood of mice infected with SeV (Fig. 2*E*). Thus, the ability of SeV but not LPS to induce FcεRI expression was related to the preferential accumulation of CD49d^{+} PMNs in the lungs of SeV-infected mice.

PMN CD11b mediates induction of FcεRI on lung cDCs

We next wanted to determine what proteins were involved in the cognate interaction between lung cDCs and PMNs. One likely target was CD49d; however, the CD49d mAb used for cell sorting PMNs is a blocking Ab (19). Therefore, because flow-sorted CD49d^{+} PMNs induced FcεRIα expression on lung cDCs, CD49d must not be directly involved in the induction of FcεRIα expression on cDCs. We next focused on two ligands for DC-specific ICAM-3–grabbing nonintegrin (DC-SIGN) that have been implicated in interactions between PMNs and cDCs: CD11b and CEACAM-1 (20, 21). Culturing PMNs with a blocking mAb to CD11b preaddition to cDCs significantly inhibited induction of FcεRI by 80% (Fig. 3). Blocking CEACAM-1 on PMNs did not affect FcεRI expression, nor did addition of an anti–DC-SIGN mAb to the cDC (data not shown). Because we demonstrated previously that naive lung cDCs do not express CD11b, we believe the effects of anti-CD11b were mediated through blockade of CD11b on the PMNs (9). CD11b expression is not restricted to the CD49d^{+} PMN subset, and in fact, the CD49d^{-} PMNs have greater expression of CD11b than CD49d^{+} PMNs (fold mean fluorescence intensity [MFI] of 202 $\pm$ 30 versus 72 $\pm$ 3, respectively; $p < 0.003$; $n = 5$ mice per group); therefore, even the modest level of CD11b expression is sufficient to mediate the PMN–cDC interaction, although additional mechanisms must be involved in induction of FcεRI on lung cDCs. Moreover, it is not surprising that the induction of FcεRI on the cDC should involve mechanisms outside the reported interactions among CD11b, CEACAM-1, and DC-SIGN, as those have been reported to lead to a Th1-mediated response, not a Th2-directed one (20–22). Our current studies are focused on understanding these other factors involved in this PMN–cDC interaction.

In summary, our data are the first, to our knowledge, to show SeV infection specifically increased the accumulation of CD49d^{+} PMNs in the lung, which in turn induced cDCs to express FcεRI. Furthermore, the increased expression of FcεRI on cDCs required the cognate interaction of CD49d^{+} PMNs with cDCs, a process dependent primarily on the expression of CD11b on the PMNs. These unexpected results implicate CD49d^{+} PMNs as an essential effector cell in the induction of chronic asthma induced by viral infection. These results suggest that one focus of future therapeutic attempts to block translation of viral illness into atopic disease should be the CD49d^{+} PMN population.

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Disclosures

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References

1. Asher, M. I., S. Montefort, B. Björkstén, C. K. Lai, D. P. Strachan, S. K. Weiland, and H. Williams; ISAAC Phase Three Study Group. 2006. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 368: 733–743.
2. Khan, S. H., S. S. Park, I. A. Sirajuddin, and M. H. Grayson. 2008. Respiratory virus and asthma: the role of immunoglobulin E. *Clin. Ther.* 30(Spec No): 1017–1024.
3. Cookson, W. O., and M. F. Moffatt. 1997. Asthma: an epidemic in the absence of infection? *Science* 275: 41–42.
4. Sigurs, N. 2001. Epidemiologic and clinical evidence of a respiratory syncytial virus-reactive airway disease link. *Am. J. Respir. Crit. Care Med.* 163: S2–S6.
5. Sigurs, N. 2002. A cohort of children hospitalised with acute RSV bronchiolitis: impact on later respiratory disease. *Paediatr. Respir. Rev.* 3: 177–183.
6. Johnston, S. L., P. K. Pattemore, G. Sanderson, S. Smith, M. J. Campbell, L. K. Josephs, A. Cunningham, B. S. Robinson, S. H. Myint, M. E. Ward, et al. 1996. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am. J. Respir. Crit. Care Med.* 154: 654–660.

7. Walter, M. J., J. D. Morton, N. Kajiwara, E. Agapov, and M. J. Holtzman. 2002. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J. Clin. Invest.* 110: 165–175.
8. Grayson, M. H., D. Cheung, M. M. Rohlfing, R. Kitchens, D. E. Spiegel, J. Tucker, J. T. Battaile, Y. Alevy, L. Yan, E. Agapov, et al. 2007. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J. Exp. Med.* 204: 2759–2769.
9. Grayson, M. H., M. S. Ramos, M. M. Rohlfing, R. Kitchens, H. D. Wang, A. Gould, E. Agapov, and M. J. Holtzman. 2007. Controls for lung dendritic cell maturation and migration during respiratory viral infection. *J. Immunol.* 179: 1438–1448.
10. Raptis, S. Z., S. D. Shapiro, P. M. Simmons, A. M. Cheng, and C. T. Pham. 2005. Serine protease cathepsin G regulates adhesion-dependent neutrophil effector functions by modulating integrin clustering. *Immunity* 22: 679–691.
11. Akk, A. M., P. M. Simmons, H. W. Chan, E. Agapov, M. J. Holtzman, M. H. Grayson, and C. T. Pham. 2008. Dipeptidyl peptidase I-dependent neutrophil recruitment modulates the inflammatory response to Sendai virus infection. *J. Immunol.* 180: 3535–3542.
12. Tate, M. D., Y. M. Deng, J. E. Jones, G. P. Anderson, A. G. Brooks, and P. C. Reading. 2009. Neutrophils ameliorate lung injury and the development of severe disease during influenza infection. *J. Immunol.* 183: 7441–7450.
13. Fleming, T. J., M. L. Fleming, and T. R. Malek. 1993. Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J. Immunol.* 151: 2399–2408.
14. Schlueter, A. J., T. R. Malek, C. N. Hostetler, P. A. Smith, P. deVries, and T. J. Waldschmidt. 1997. Distribution of Ly-6C on lymphocyte subsets: I. Influence of allotype on T lymphocyte expression. *J. Immunol.* 158: 4211–4222.
15. Asselin-Paturel, C., G. Brizard, J. J. Pin, F. Brière, and G. Trinchieri. 2003. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. *J. Immunol.* 171: 6466–6477.
16. Megiovanni, A. M., F. Sanchez, M. Robledo-Sarmiento, C. Morel, J. C. Gluckman, and S. Boudaly. 2006. Polymorphonuclear neutrophils deliver activation signals and antigenic molecules to dendritic cells: a new link between leukocytes upstream of T lymphocytes. *J. Leukoc. Biol.* 79: 977–988.
17. Tsuda, Y., H. Takahashi, M. Kobayashi, T. Hanafusa, D. N. Herndon, and F. Suzuki. 2004. Three different neutrophil subsets exhibited in mice with different susceptibilities to infection by methicillin-resistant *Staphylococcus aureus*. *Immunity* 21: 215–226.
18. Sullivan, G. W., D. D. Lee, W. G. Ross, J. A. DiVietro, C. M. Lappas, M. B. Lawrence, and J. Linden. 2004. Activation of A2A adenosine receptors inhibits expression of alpha 4/beta 1 integrin (very late antigen-4) on stimulated human neutrophils. *J. Leukoc. Biol.* 75: 127–134.
19. Kamata, T., W. Puzon, and Y. Takada. 1995. Identification of putative ligand-binding sites of the integrin alpha 4 beta 1 (VLA-4, CD49d/CD29). *Biochem. J.* 305: 945–951.
20. van Gisbergen, K. P., I. S. Ludwig, T. B. Geijtenbeek, and Y. van Kooyk. 2005. Interactions of DC-SIGN with Mac-1 and CEACAM1 regulate contact between dendritic cells and neutrophils. *FEBS Lett.* 579: 6159–6168.
21. van Gisbergen, K. P., M. Sanchez-Hernandez, T. B. Geijtenbeek, and Y. van Kooyk. 2005. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J. Exp. Med.* 201: 1281–1292.
22. Bennouna, S., S. K. Bliss, T. J. Curiel, and E. Y. Denkers. 2003. Cross-talk in the innate immune system: neutrophils instruct recruitment and activation of dendritic cells during microbial infection. *J. Immunol.* 171: 6052–6058.