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Brief report

Deficient MHC class I cross-presentation of soluble antigen by murine neonatal dendritic cells

Tobias R. Kollmann, Sing Sing Way, Heidi L. Harowicz, Adeline M. Hajjar, and Christopher B. Wilson

Neonates respond suboptimally to many vaccines. The reasons for this defect are unclear, but suboptimal antigen presentation by dendritic cells has been suggested as one possibility. In this report we describe an *in vitro* system that allows the generation of large numbers of resting murine neonatal dendritic cells facili-

tating their study. Using this system, we show a clear reduction in the ability of neonatal dendritic cells to present soluble ovalbumin, while the capacity to present ovalbumin peptide is intact. This suggests a specific defect in cross-presentation of exogenous antigen via the major histocompatibility complex

(MHC) class I pathway. Deficient cross-presentation may contribute to the suboptimal CD8 T-cell response to vaccines in neonates. (*Blood*. 2004;103:4240-4242)

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Introduction

The induction of antigen-specific CD8⁺ cytotoxic T lymphocytes (CTLs) by vaccines is reduced in neonates compared with adults.^{1,2} However, this deficit can be overcome if the antigen is introduced into or produced within the cytoplasm of neonatal dendritic cells (DCs).^{2,3} Neonates also generate CTLs if the antigen is given as processed peptide.³ This suggests a defect in the neonates' ability to process and present exogenous antigens via the major histocompatibility complex (MHC) class I pathway, which is known as cross-presentation.^{4,5} The pathways for cross-presentation of soluble antigens versus cell-associated antigens are known to be different.^{6,7} Cross-presentation by neonatal DCs of soluble antigens has not been investigated. As this is relevant to the design of vaccines for neonates, we wished to establish an *in vitro* system amenable to experimental manipulation. Using this system, we set out to test our primary hypothesis that cross-presentation of soluble antigens by neonatal DCs is less efficient than that by adult DCs.

Study design

DCs were isolated directly *ex vivo* from neonatal (5-day-old) or adult mouse spleens by positive selection using anti-CD11c magnetic beads.³ To generate DCs *in vitro*, adult bone marrow and neonatal (< 24 hours) liver from C57BL/6 mice were used as the richest source of hematopoietic precursor cells.⁸ Single-cell suspensions of these organs were cultured in medium containing granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN) or Flt-3 ligand (Flt-L; Amgen, Thousand Oaks, CA), as described.⁹ When indicated, DCs were stimulated with polyinosinic-polycytidylic acid (pI:C; 50 µg/mL; Amersham, Arlington Heights, IL) or lipopolysaccha-

ride (LPS; 100 ng/mL *Salmonella* minnesota Re595; Sigma-Aldrich, St Louis, MO). CD8⁺ T cells were isolated from the spleens of ovalbumin (OVA) T-cell receptor (TCR)-transgenic (OTI) mice¹⁰ on a C57BL/6 background by positive selection using anti-CD8 magnetic beads after depletion of CD11c⁺ cells. OVA-specific (Sigma; grade VI, 99% pure, 50 µg/mL) and OVA peptide-specific (SIINFEKL; UBR Peptide; 10 µM) T-cell priming was detected *in vitro* as described.¹¹ Flow cytometry was performed as described.³ All mice were bred under specific pathogen-free conditions and used under the University of Washington's institutional animal care and use committee-approved protocols.

Results and discussion

We first characterized *ex vivo*, GM-CSF, or Flt-3L DCs (defined as CD11c⁺ cells) from neonatal and adult mice by flow cytometry. Overall, neonatal and adult DCs displayed a similar surface phenotype (Figure 1A), although as expected the phenotype of the 3 DC populations differed. As previously reported, CD11c⁺/B220⁺ plasmacytoid DCs were present in a higher percentage in the neonatal spleen¹²; a similar trend was observed for Flt-3L DCs (Figure 1B). We also analyzed DCs for the expression of markers relevant to presentation of antigen to CD8⁺ T cells. Neonatal or adult *ex vivo* DCs cultured short term without further stimulation shared high MHC class I and CD86 expression (Figure 1C), indicative of an activated phenotype.¹³ CD86 but not MHC class I expression was further up-regulated by stimulation with LPS or pI:C (Figure 1C), confirming that the regulation of MHC class I surface expression differs from that of CD86.¹⁴ Unstimulated *ex vivo* neonatal DCs expressed more CD86 than adult DCs, but similar amounts after activation.

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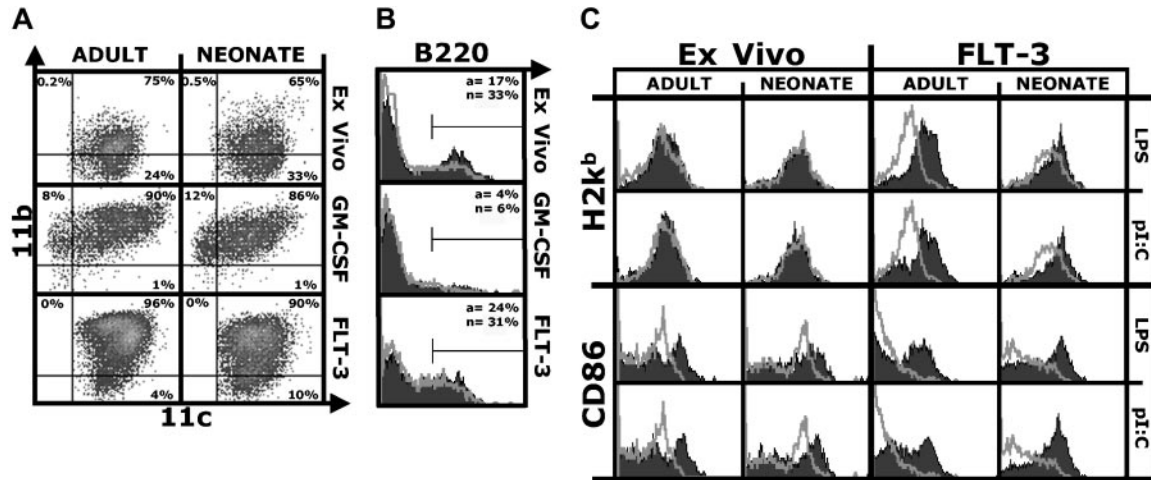


Figure 1. Similar surface phenotype of adult and neonatal DCs. (A) CD11b/CD11c density plot of DCs isolated from spleens of adult or neonatal mice (ex vivo) or derived by culturing precursors in GM-CSF or Flt-3L. Acquisition gates were set to exclude debris and dead cells, and quadrants for analysis were set based on control isotype-stained samples. Percentage of positive cells is indicated for each quadrant. (B) B220 expression on neonatal (black filled) or adult (gray line) DCs. Percentage of positive cells for adults (a) or neonates (n) is indicated. (C) H2K^b (MHC-I) and CD86 expression on ex vivo or Flt-3L neonatal and adult DCs after short-term culture (18 hours) without (gray line) or with (black filled) LPS (100 ng/mL) or pI:C (50 μg/mL). The figures show one representative experiment of 3.

Compared with ex vivo DCs, Flt-3L DCs had a resting phenotype—they expressed low amounts of MHC class I and CD86. Both adult and neonatal Flt-3L DCs up-regulated MHC class I and CD86 in response to LPS or pI:C (Figure 1C). Results with resting and stimulated neonatal and adult GM-CSF DCs were similar (not shown). Overall, there was little or no difference between neonatal and adult DCs in MHC class I, CD86 (Figure 1), CD80, and CD40 (not shown) expression.

To analyze cross-presentation of soluble antigen, we compared DCs in their capacity to stimulate OVA-specific (OTI) CD8⁺ T cells from adult mice, using OVA peptide as a positive control for

direct presentation not requiring antigen processing. While both neonatal and adult DCs induced the proliferation of OTI cells after loading with OVA peptide, neonatal DCs displayed a clearly reduced capacity to process and present soluble OVA (Figure 2). This did not reflect a difference in the optimal concentration of soluble OVA, since preliminary experiments demonstrated that 50 μg/mL induced maximal proliferation of OTI cells cultured with adult or neonatal DCs (data not shown). This deficit was evident with ex vivo, Flt-3L, and GM-CSF neonatal DCs. Cross-presentation of cell-associated antigen by neonatal DCs and of cell-associated or soluble antigens by adult DCs can be enhanced by inflammatory stimuli.¹⁵⁻¹⁸ In our system, the proinflammatory stimuli LPS and pI:C enhanced cross-presentation of soluble OVA by adult but not neonatal DCs (Figure 2).

Uptake of fluorescein isothiocyanate (FITC)-labeled OVA was equally efficient in neonatal and adult DCs (data not shown) as previously shown,³ and thus is unlikely to be the limiting factor in cross-presentation for neonatal DCs. Peptide presentation on the cell surface also appears intact, as the proliferation of OTI T cells was induced equally by neonatal and adult DCs loaded with OVA peptide (Figure 2, peptide; Dadaglio et al³). Thus, the difference between the neonatal and adult DCs in their ability to cross-present soluble antigen must lie in the processing and/or transport pathways between phagosome and cytosol.^{19,20} For adult in vitro-derived DCs, soluble OVA is known to be sequestered intracellularly after uptake, and to enter the cross-presentation pathway only after receiving other signals.²¹ The exact nature of these signals is unknown, and may differ between adult and neonatal DCs. It remains to be determined where in these complex pathways the defect in the neonatal DC lies: in the pathway of antigen processing, signal transduction, or both. Our data show a clear reduction in cross-presentation of soluble antigen in neonatal DCs. This defect was not overcome by inflammatory stimuli, and may contribute to the neonate's reduced generation of CTLs in response to vaccines.

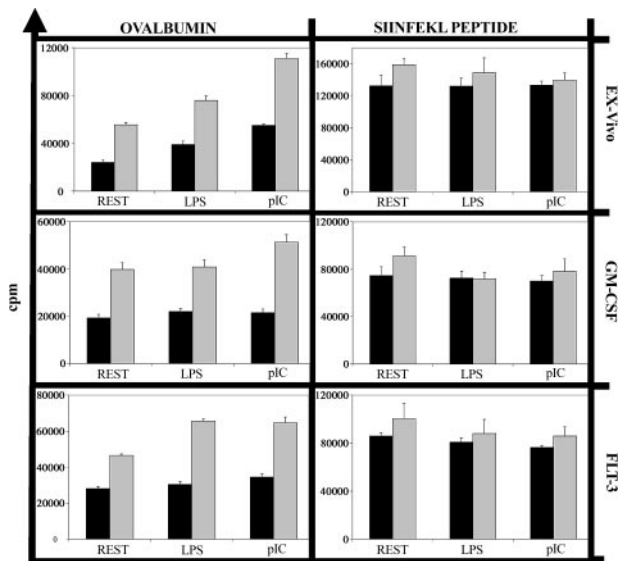


Figure 2. Deficient cross-presentation of soluble ovalbumin by neonatal DCs. Neonatal (■) and adult (□) DCs (1×10^4 cells) were incubated with soluble ovalbumin (50 μg/mL) or the MHC class I-restricted ovalbumin peptide SIINFEKL (10 μM) along with nothing (REST), LPS (100 ng/mL), or pI:C (50 μg/mL) for 18 hours. After 3 washes, purified adult OTI CD8⁺ T cells (1×10^5 cells) were added, and after 48 hours cultures were pulsed with 5 μCi (0.185 MBq) ³H-thymidine for 18 hours. Background proliferation of DCs alone, T cells alone, or DCs plus T cells but without ovalbumin or peptide was always less than 1000 counts per minute (cpm). Error bars indicate the standard error of the mean (SEM) of triplicate samples for this experiment. The graphs show one representative experiment of 3.

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References

- Siegrist CA. Neonatal and early life vaccinology. *Vaccine*. 2001;19:3331-3346.
- Siegrist CA, Sadhalla F, Tougne C, Martinez X, Kovarik J, Lambert PH. Induction of neonatal Th1 and CTL responses by live viral vaccines: a role for replication patterns within antigen presenting cells? *Vaccine*. 1998;16:1473-1478.
- Dadaglio G, Sun CM, Lo-Man R, Siegrist CA, Leclerc C. Efficient in vivo priming of specific cytotoxic T cell responses by neonatal dendritic cells. *J Immunol*. 2002;168:2219-2224.
- Belz GT, Carbone FR, Heath WR. Cross-presentation of antigens by dendritic cells. *Crit Rev Immunol*. 2002;22:439-448.
- Lizee G, Basha G, Tiang J, et al. Control of dendritic cell cross-presentation by the major histocompatibility complex class I cytoplasmic domain. *Nat Immunol*. 2003;4:1065-1073.
- Fonteneau JF, Kavanagh DG, Lirvall M, et al. Characterization of the MHC class I cross-presentation pathway for cell associated antigens by human dendritic cells. *Blood*. Prepublished on August 21, 2003, as DOI 10.1182/blood-2003-06-1801.
- Li M, Davey GM, Sutherland RM, et al. Cell-associated ovalbumin is cross-presented much more efficiently than soluble ovalbumin in vivo. *J Immunol*. 2001;166:6099-6103.
- Bunting KD, Bradley HL, Hawley TS, Moriggl R, Sorrentino BP, Ihle JN. Reduced lymphomyeloid repopulating activity from adult bone marrow and fetal liver of mice lacking expression of STAT5. *Blood*. 2002;99:479-487.
- Boonstra A, Asselin-Paturel C, Gilliet M, et al. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. *J Exp Med*. 2003;197:101-109.
- Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR. T cell receptor antagonist peptides induce positive selection. *Cell*. 1994;76:17-27.
- Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. *J Exp Med*. 2002;195:1653-1659.
- Sun CM, Fiette L, Tanguy M, Leclerc C, Lo-Man R. Ontogeny and innate properties of neonatal dendritic cells. *Blood*. 2003;102:585-591.
- Edwards AD, Diebold SS, Slack EM, et al. Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8α⁺ DC correlates with unresponsiveness to imidazoquinolines. *Eur J Immunol*. 2003;33:827-833.
- Honda K, Sakaguchi S, Nakajima C, et al. Selective contribution of IFN-α/β signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. *Proc Natl Acad Sci U S A*. 2003;100:10872-10877.
- Hoglund, P, Mintern J, Waltzinger C, Heath W, Benoist C, Mathis D. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. *J Exp Med*. 1999;189:331-339.
- Mintern JD, Sutherland RM, Lew AM, Shortman K, Carbone FR, Heath WR. Constitutive, but not inflammatory, cross-presentation is disabled in the pancreas of young mice. *Eur J Immunol*. 2002;32:1044-1051.
- Heit A, Maurer T, Hochrein H, et al. Toll-like receptor 9 expression is not required for CpG DNA-aided cross-presentation of DNA-conjugated antigens but essential for cross-priming of CD8 T cells. *J Immunol*. 2003;170:2802-2805.
- Datta SK, Redecke V, Prilliman KR, et al. A subset of Toll-like receptor ligands induces cross-presentation by bone marrow-derived dendritic cells. *J Immunol*. 2003;170:4102-4110.
- Guermonprez P, Savenau L, Kleijmeier M, Davoust J, Van Ender P, Amigorena S. ER-phagosome fusion defines MHC class I cross-presentation compartment in dendritic cells. *Nature*. 2003;425:397-402.
- Houde M, Bertholet S, Gagnon E, et al. Phagosomes are competent organelles for antigen cross-presentation. *Nature*. 2003;425:402-406.
- Delamarre L, Holcombe H, Mellman I. Presentation of exogenous antigens on major histocompatibility complex (MHC) class I and MHC class II molecules is differentially regulated during dendritic cell maturation. *J Exp Med*. 2003;198:111-122.