	K	keratinocyte cell line on exposure to Propionibacterium acnes.					
31	Suzuki K, Hirokawa K, Yagami A, Matsunaga K	Allergic Contact Dermatitis from Carmine in Cosmetic Blush	Dermatitis	22 (6)	348-349	2011	
32	Sasaki R, Suzuki K, Hayashi T, Inasaka H, Matsunaga K	Improvement of Chelitis granulomatosa after Dental Treatment	Case Rep Dermatol	3	151-154	2011	*
33	Suzuki K, Yagami A, Matsunaga K	Allergic Contact Dermatitis caused by a skin-lightening agent, 5,5'-dipropylbipheny1-2,2'-diol	Contact Dermatitis	66	46-52	2011	
34	Fukamachi S, Mori T, Sakabe JI, Shiraishi N, Kuroda E, Kobayashi M, Bito T, Kabashima K, Nakamura M, Tokura Y	Topical cholecystokinin depresses itch-associated scratching behavior in mice.	J Invest Dermatol	131	956-961	2011	*
35	Fukamachi S, Bito T, Shiraishi N, Kobayashi M, Kabashima K, Nakamura M, Tokura Y	Modulation of semaphorin 3A expression by calcium concentration and histamine in human keratinocytes and fibroblasts.	J Dermatol Sci	61	118-123	2011	*
36	Sugita K, Kabashima K, Sawada Y, Haruyama S, Yoshioka M, Mori T, Ogasawara K, Tokura Y	Blocking of CTLA-4 on lymphocytes improves the sensitivity of lymphocyte transformation test in a patient with nickel allergy.	Eur J Dermatol		(in press)		*
37	Sawada Y, Hino R, Hama K, Ohmori S, Fueki H, Yamada S, Fukamachi S, Tajiri M, Kumo R, Yoshioka M, Nakashima D, Sugita	The type of skin eruptions is an independent prognostic indicator for adult T-cell leukemia/lymphoma.	Blood	117	3961-3967	2011	*

	K, Yoshiki R, Shimauchi T, Mori T, Izu K, Kobayashi M, Nakamura M, Tokura Y						
38	Kobayashi M, Tokura Y	Downmodulatory effects of cetirizine and levocetirizine on cytokine/chemokine production and CD54 expression in keratinocytes.	J Dermatol	38	717-719	2011	
39	Hino R, Kabashima R, Kawakami C, Sugita K, Nakamura M, Tokura Y	Circulating Th17 cell fluctuation in psoriatic patients treated with topical calcipotriol and betamethasone butyrate propionate.	J Eur Acad Dermatol Venereol	25	242-244	2011	
40	Park K, Mori T, Nakamura M, Tokura Y	Increased expression of mRNAs for IL-4, IL-17, IL-22 and IL-31 in skin lesions of subacute and chronic forms of prurigo.	Eur J Dermatol	21	135-136	2011	
41	Ohmori S, Nakamura M, Hino R, Kobayashi M, Tokura Y	Inflammatory cytokine expression in the skin lesions of tumour necrosis factor receptor-associated periodic syndrome.	Rheumatology	51	196-199	2012	*
42	Honda T, Otsuka A, Tanizaki H, Minegaki Y, Nagao K, Waldmann H, Tomura M, Hori S, Miyachi Y, Kabashima K	Enhanced murine contact hypersensitivity by depletion of endogenous regulatory T cells in the sensitization phase.	J Dermatol Sci	61	144-7	2011	
43	Otsuka A, Kubo M, Honda T, Egawa G, Nakajima S, Tanizaki H, Kim B, Matsuoka S, Watanabe T, Nakae S,	Requirement of Interaction between Mast Cells and Skin Dendritic Cells to Establish Contact Hypersensitivity.	PLoS One	6(9)	e25538	2011	*

	Miyachi Y, Kabashima K						
44	Nakahigashi K, Doi H, Otsuka A, Hirabayashi T, Murakami M, Urade Y, Tanizaki H, Egawa G, Miyachi Y, Kabashima K	PGD(2) induces eotaxin-3 via PPARγ from sebocytes: A possible pathogenesis of eosinophilic pustular folliculitis.	J Allergy Clin Immunol		(in press)	2011	*
45	Otsuka A, Honda T, Doi H, Miyachi Y, Kabashima K	An H1-histamine receptor antagonist decreases serum interleukin-31 levels in patients with atopic dermatitis.	Br J Dermatol.	164	455-6	2011	
46	Egawa G, Honda T, Tanizaki H, Doi H, Miyachi Y, Kabashima K	In vivo imaging of T-cell motility in the elicitation phase of contact hypersensitivity using two-photon microscopy.	J Invest Dermatol.	131	977-9.	2011	
47	Nakahigashi K, Kabashima K, Ikoma A, Verkman AS, Miyachi Y, Hara-Chikuma M	Upregulation of aquaporin-3 is involved in keratinocyte proliferation and epidermal hyperplasia.	J Invest Dermatol.	131	865-73	2011	
48	Otsuka A, Miyagawa-Hayashino A, Walls A, Miyachi Y, Kabashima K	Comparison of basophil infiltration into the skin between eosinophilic pustular folliculitis and neutrophilic folliculitis.	J Eur Acad Dermatol Venereol.		(in press)	2011	
49	Honda T, Miyachi Y, Kabashima K	Regulatory T cells in cutaneous immune responses.	J Dermatol Sci.	63	75-82	2011	
50	Hara-Chikuma M, Sugiyama Y, Kabashima K, Sohara E, Uchida S, Sasaki S, Inoue S, Miyachi Y.	Involvement of aquaporin-7 in the cutaneous primary immune response through modulation of antigen uptake and migration in	FASEB J.	26	211-218	2012	

		dendritic cells.					
51	川野光子、小笠原康悦	金属アレルギーの免疫学 Up-to-date.	Visual Dermatology	10 (11)	1208-1212	2011	
52	平澤典保、大内和雄	増悪化アレルギー性皮膚炎症におけるヒスタミンの役割	薬学雑誌	131	179-184	2011	
53	平澤典保	ステロイド薬の基礎	アレルギー	60	193-198	2011	
54	平澤典保、Hong JangJa、木邑道夫、大内和雄	TSLP	炎症と免疫	19	437-440	2011	
55	平澤典保、Hong JangJa、木邑道夫、大内和雄	抗炎症薬	JOHNS	27	1782-1786	2011	
56	平澤典保、奥直人	薬学新時代の大学院教育	薬学雑誌	131	931	2011	
57	富岡佳久、平澤典保、永沼章	6 年制の大学院教育 : 東北大学の場合	薬学雑誌	131	932-938	2011	
58	平澤典保	キシレン、トリメチルベンゼンによる表皮からの TSLP 産生誘導	臨床と免疫・アレルギー科	56	478-484	2011	
59	平澤典保	ステロイドの作用機序と耐性化メカニズム	アレルギーの臨床	31	1137–1141	2011	
60	平澤典保	ステロイド薬の基礎 適切な使用のために	小児科	53	203–210	2011	
61	鈴木加余子, 松永佳世子	パッチテストアレルゲンに関するアンケート 2010	Journal of Environmental Dermatology and Cutaneous Allergology	5 (2)	91-102	2011	*
62	矢上晶子、松永佳世子	パッチテストの基本手技と解釈	医学のあゆみ	240 (3)	249	2012	
63	戸倉新樹	2 つのアトピー性皮膚炎 : 外因性と内因性.	皮膚アレルギーフロンティア	9	60-63	2011	
64	戸倉新樹	二つのアトピー性皮膚炎.	日本香粧品	35	19-22	2011	

			学会誌				
65	戸倉新樹	外因性と内因性という区分けはアトピー性皮膚炎に必要か？	皮膚の科学	10	34-37	2011	
66	坂部純一，戸倉新樹	皮膚バリア機能を担うフィラグリンの成熟機構.	Visual Dermatology	10	1272-1274	2011	

書籍

（欧文）

	著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	ページ	出版年	報告書に掲載
1	Ohtsu H	Histamine synthesis and lessons learned from histidine decarboxylase deficient mice	Robin L. Thurmond	Histamine in inflammation	Landes Bioscience	Austen Texas	21-31	2011	

（和文）

	著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	ページ	出版年	報告書に掲載
2	矢上晶子、松永佳世子	既成パッチテストテープはどこまで使えるか	宮地良樹	What's new in 皮膚科学 2012-201	株式会社メディカルレビュ	東京	46-47	2012	*

				3	一社				
3	戸倉新樹	アトピー性皮膚炎における獲得免疫の異常.	古江増隆, 中村晃一郎編.	皮膚科臨床アセット 1. アトピー性皮膚炎.	中山書店.		130-135	2011	
4	椛島健治	皮膚科との接点	清野宏	臨床粘膜免疫学	シナジー	東京	681-691	2010	
5	椛島健治	脂質メディエーターと皮膚免疫･アレルギー疾患	横溝岳彦	脂質生物学（実験医学増刊）	羊土社	東京	3395-3340	2010	
6	本田哲也、椛島健治	樹状細胞	戸倉新樹	ファーストステップ皮膚免疫学	中外医学社	東京	11-22	2010	
7	江川形平、椛島健治	接触皮膚炎の免疫学的メカニズム	戸倉新樹	ファーストステップ皮膚免疫学	中外医学社	東京	99-105	2010	
8	松永佳世子ほか	最新・歯科と連携して治す皮膚疾患	松永佳世子	Visual Dermatology	秀潤社	東京	1115-1240	2011	*

V. 研究成果の刊行物・別刷

Natural killer (NK)–dendritic cell interactions generate MHC class II-dressed NK cells that regulate CD4$^+$ T cells

Masafumi Nakayama[a], Kazuyoshi Takeda[b], Mitsuko Kawano[a], Toshiyuki Takai[c], Naoto Ishii[d], and Kouetsu Ogasawara[a,1]

Departments of [a]Immunobiology and [c]Experimental Immunology, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980-8575, Japan; [b]Department of Immunology, Juntendo University School of Medicine, Tokyo 113-8421, Japan; and [d]Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan

Edited* by Lewis L. Lanier, University of California, San Francisco, CA, and approved October 11, 2011 (received for review July 1, 2011)

Natural killer (NK) cells contribute to not only innate but also to adaptive immunity by interacting with dendritic cells (DCs) and T cells. All activated human NK cells express HLA-DR and can initiate MHCII-dependent CD4$^+$ T-cell proliferation; however, the expression of MHCII by mouse NK cells and its functional significance are controversial. In this study, we show that NK–DC interactions result in the emergence of MHCII-positive NK cells. Upon in vitro or in vivo activation, mouse conventional NK cells did not induce MHCII transcripts, but rapidly acquired MHCII protein from DCs. MHCII *H2-Ab1*–deficient NK cells turned I-A^b-positive when adoptively transferred into wild-type mice or when cultured with WT splenic DCs. NK acquisition of MHCII was mediated by intercellular membrane transfer called "trogocytosis," but not upon DAP10/12- and MHCI-binding NK cell receptor signaling. MHCII-dressed NK cells concurrently acquired costimulatory molecules such as CD80 and CD86 from DCs; however, their expression did not reach functional levels. Therefore, MHCII-dressed NK cells inhibited DC-induced CD4$^+$ T-cell responses rather than activated CD4$^+$ T cells by competitive antigen presentation. In a mouse model for delayed-type hypersensitivity, adoptive transfer of MHCII-dressed NK cells attenuated footpad swelling. These results suggest that MHCII-dressed NK cells generated through NK–DC interactions regulate T cell-mediated immune responses.

Natural killer (NK) cells have long been known to play important roles in innate immunity, but recently their contributions to adaptive immunity have also been reported (1). Following immunization or infection, NK cells migrate to draining lymphoid organs, where they interact with dendritic cells (DCs) and/or T cells (2). By production of cytokines such as IFN-γ and TNF-α and direct cell–cell contact, NK cells activate DCs to induce T-cell proliferation and differentiation (3, 4). However, NK cells can also negatively regulate adaptive immune responses (2, 5). For example, in mouse models of autoimmune diseases such as rheumatoid arthritis and experimental autoimmune encephalomyelitis, depletion of NK cells by anti-NK1.1 mAb exacerbates these diseases (5). However, the molecular mechanisms underlying negative immune regulation by NK cells are poorly understood (5). Although conventional mouse NK cells do not express MHCII, subpopulations of activated mouse NK cells have been found to express MHCII (6–9), suggesting that NK cells may directly regulate CD4$^+$ T-cell responses. Of note, activated human NK cells express HLA-DR and can induce MHCII-dependent CD4$^+$ T-cell proliferation (6, 10–13).

MHCII molecules are crucial for the presentation of peptides processed from extracellular proteins to CD4$^+$ T cells, and they shape T-cell receptor repertoire development during T-cell maturation and lineage commitment. The constitutive expression of MHCII is restricted to professional antigen-presenting cells (APCs) such as DCs, macrophages, and B cells (14). In addition to professional APCs, basophils also express MHCII and play a crucial role as APCs (15). MHCII expression is transcriptionally regulated by the class II transactivator (CIITA) in APCs, including basophils (14, 15). However, the regulation of MHCII expression in murine NK cells and the mechanism by which MHCII$^+$ NK cells are generated remain unclear.

A wide variety of immune cell lineages communicate with each other through direct cell–cell contact or cytokine production to establish appropriate immune responses. Several recent studies have shown that during direct cell–cell interactions, plasma membrane fragments of one cell are transferred to the opposite cell (16, 17). This phenomenon is currently called "trogocytosis" (17, 18), and it may generate novel cell populations that result from the interaction between two different types of immune cells. Indeed, Wakim and Bevan have recently reported that DC–DC interactions generate a novel DC subset, called "cross-dressed" DCs, which acquire peptide–MHCI complexes from donor DCs to drive memory CD8$^+$ T-cell activation (19). Therefore, immune responses can be regulated not only by lineage-committed cell populations but also by cell populations generated independently of transcription, by trogocytosis. Whether NK–DC interactions produce novel cell populations in a similar manner has not been explored. Therefore, we investigated whether MHCII-positive NK cells could be generated through interactions between mouse conventional NK cells and splenic DCs, and asked whether the resulting MHCII-positive NK cells could regulate CD4$^+$ T-cell responses.

Results

Activated NK Cells Express MHCII Protein, Although Not the Transcript, in Vivo. MHCII expression has been observed on human NK cells prepared from mixed lymphocyte cultures or pathogen-infected organs, suggesting that activated NK cells induce MHCII (10, 11). Consistent with these reports, we observed MHCII I-A^b expression on splenic NK1.1$^+$ cells in C57BL/6 mice injected i.v. with the double-stranded RNA synthetic analog polyI:C, but not in naïve mice (Fig. 1*A*). These NK1.1$^+$ cells also expressed NKG2D and DX5 (Fig. 1*A*), indicating that these cells were conventional NK cells. Expression levels of MHCII on splenic B cells and T cells were not altered by polyI:C administration (Fig. S1). To confirm that I-A^{b+} NK1.1$^+$ cells were a single NK cell population and not merely conjugates of NK cells and I-A^{b+} cells such as DCs, we sort-purified these cells and analyzed them by

Author contributions: M.N. and K.O. designed research; M.N. performed research; T.T. and N.I. contributed new reagents/analytic tools; K.T., M.K., and K.O. analyzed data; and M.N., K.T., and K.O. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: ogasawara@idac.tohoku.ac.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1110584108/-/DCSupplemental.

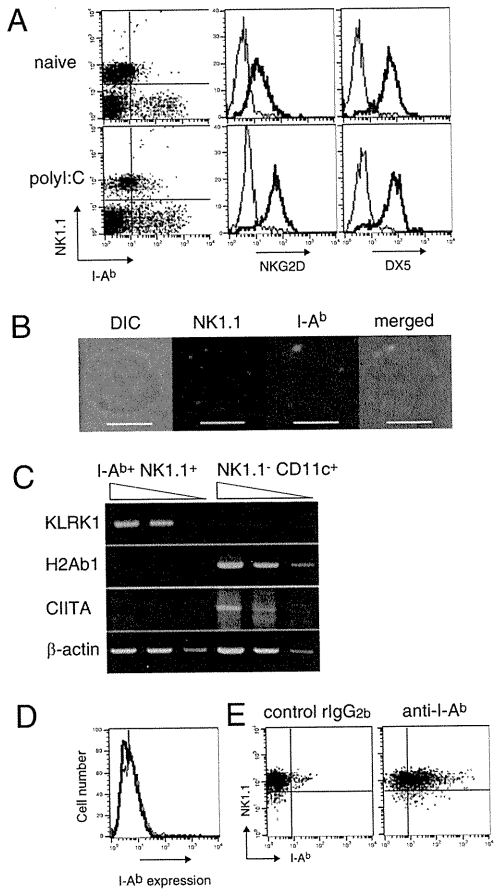

Fig. 1. Activated NK cells express MHCII protein but not the transcript in vivo. (*A*) NK1.1 and I-A^b expression on a non-T/B-cell population in spleen from naive or polyI:C (100 μg per mouse)-treated mice was analyzed (*Left*). Expression of NKG2D (*Center*) and DX5 (*Right*) on $NK1.1^+$ cells from splenocytes was analyzed using isotype control mAbs (thin lines) and specific mAbs (thick lines). (*B*) Purified I-Ab^+ $DX5^+$ cells were stained by AF488-anti–I-A^b mAb and biotinylated anti-NK1.1 mAb, followed by DyLight 594-streptavidin. (Scale bars, 5 μm.) (*C*) Expression of the indicated transcripts in purified splenic I-Ab^+ $NK1.1^+$ cells or $NK1.1^-$ $CD11c^+$ cells was analyzed by semiquantitative RT-PCR using 10-fold serially diluted cDNA templates. (*D*) Splenic NK cells were purified and then cultured with IL-2 (1,000 U/mL) for 5 d, and then stained with control rat IgG_{2b} (thin line) or anti–I-A^b mAb (thick line). (*E*) NK cells cultured as described in *D* were labeled with CFSE and then transferred into mice. The following day, I-A^b and NK1.1 expression level on $CFSE^+$ cells in spleen was analyzed. Similar results were obtained in three (*A*, *D*, and *E*) or two (*B* and *C*) independent experiments.

confocal microscopy. Fig. 1*B* shows that $NK1.1^+$ cells per se express I-A^b on their cell surface.

To explore whether I-A^b expression on NK cells depended on transcriptional regulation, we next performed semiquantitative PCR on mRNA from sort-purified NK cells. Unexpectedly, we observed neither MHCII *H2-Ab1* transcript nor the transactivator *CIITA* transcript in I-A^{b+} $NK1.1^+$ cells, whereas we did detect the *Klrk1* gene transcript that encodes NKG2D protein in these cells (Fig. 1*C*). Thus, it appeared that the expression of MHCII protein on NK cells occurred independently of transcriptional regulation in vivo.

We next addressed whether activated NK cells express MHCII in vitro. We purified conventional NK cells from naïve mouse spleens and cultured them with IL-2 for 5 d. Unexpectedly, these conventional NK cells remained MHCII-negative in vitro (Fig. 1*D*). Interestingly, when we labeled the cells with 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE) and transferred them into naïve mice, these NK cells turned MHCII-positive in the spleens of recipient mice (Fig. 1*E*). Nevertheless, we were unable to detect *H2-Ab1* or *CIITA* transcripts in transferred I-A^{b+} NK cells purified from the spleens of recipient mice (Fig. S2). Thus, our findings suggest that activated NK cells become MHCII-positive in vivo, and do so by transcription-independent mechanisms.

Activated NK Cells Acquire MHCII from DCs Through NK–DC Interaction. To further elucidate the pathway for $MHCII^+$ NK cell generation, we next adoptively transferred IL-2-activated MHCII *H2-Ab1* gene-deficient NK cells into wild-type (WT) mice. Surprisingly, we found that MHCII was expressed at high levels on *H2-Ab1*–deficient NK cells in WT mouse spleens within 1 d of transfer (Fig. 2*A*). In contrast, WT NK cells transferred into *H2-Ab1*–deficient mice remained MHCII-negative (Fig. 2*A*). Given that activated NK cells interact with DCs in vivo (2), we hypothesized that NK cells acquire MHCII from DCs. To address this possibility, we cocultured CFSE-labeled NK cells and splenic DCs at a 1:1 ratio. We observed that IL-2-activated NK cells turned MHCII-positive within 1 h of coculture (Fig. 2*B*). In contrast, IL-

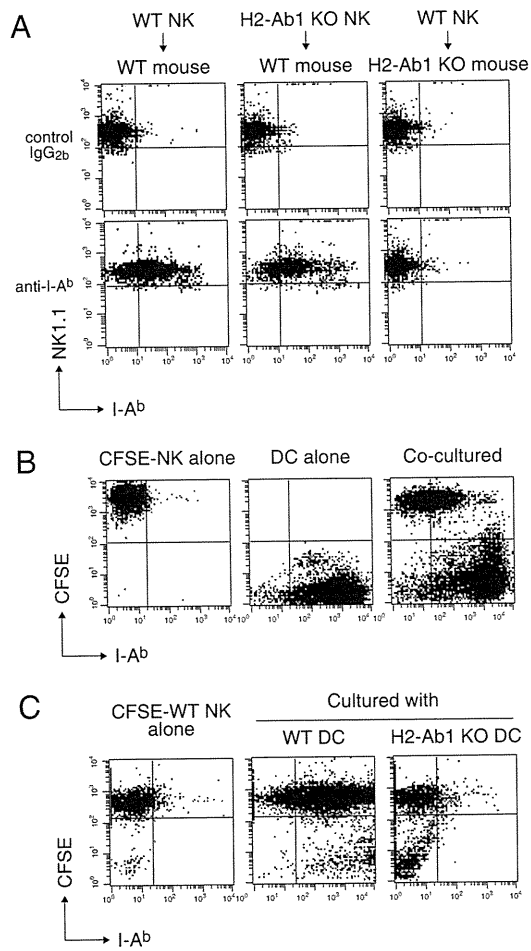

Fig. 2. Activated NK cells acquire MHCII from DCs. (*A*) WT or *H2-Ab1*–deficient NK cells prepared as described in Fig. 1*E* were adoptively transferred into WT or *H2-Ab1*–deficient mice. The following day, NK1.1 and I-A^b expression level on $CFSE^+$ cells in spleen was analyzed. (*B*) CFSE-labeled NK cells prepared as described in Fig. 1*D* were cocultured with splenic $CD11c^+$ cells at a 1:1 ratio for 1 h. I-A^b expression on these cells was analyzed. (*C*) CFSE-labeled WT NK cells were cocultured with WT or *H2-Ab1*–deficient splenic DCs, and I-A^b expression on these cells was analyzed as described in *B*. Similar results were obtained in three independent experiments.

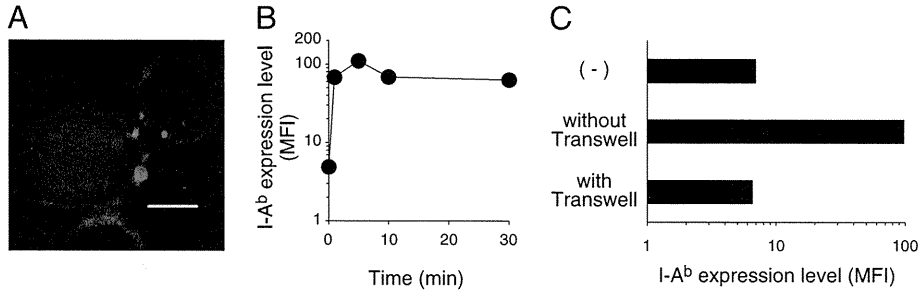

Fig. 3. Intercellular MHCII transfer is mediated by trogocytosis. (*A*) TAMRA-labeled NK cells cocultured with DCs were stained with AF488-anti–I-A^b mAb and analyzed by confocal microscopy. (Scale bar, 5 μm.) (*B*) CFSE-labeled NK cells were cocultured with DCs as described in Fig. 2*B* for the indicated periods of time. The mean fluorescence intensity (MFI) of I-A^b expression on NK cells was analyzed by flow cytometry. (*C*) CFSE-labeled NK cells were cocultured with DCs for 1 h together (no transwell) or separated by a semipermeable membrane (transwell). The MFI of I-A^b expression on NK cells was analyzed by flow cytometry. Similar results were obtained in three (*A* and *B*) and two (*C*) independent experiments.

2-activated WT NK cells remained MHCII-negative after coculture with *H2-Ab1*–deficient DCs (Fig. 2*C*), providing evidence that NK cells acquire MHCII from WT DCs. The acquired MHCII protein remained on NK cells for at least 12 h after removal of DCs (Fig. S3*A*). Freshly isolated splenic NK cells did not acquire MHCII protein from DCs effectively (Fig. S3*B*), and thus NK cells gain this ability once activated.

Intercellular protein transfer between immune cells is mediated by several pathways, including membrane nanotubes (transient long-distance connections), trogocytosis (a rapid, cell contact-dependent transfer of membrane fragment), and exosomes (secreted membrane nanovesicles of ≤100 nm) (16–18, 20). By confocal microscopy, we could not observe nanotubes between NK cells and DCs, but we found I-A^b-containing membrane fragments (~1 μm) from DCs on NK cells (Fig. 3*A*). Although DCs have been reported to secrete MHCII-containing exosomes (20), we did not detect exosomes in culture supernatants until 12 h after incubation (Fig. S4). In contrast, MHCII-containing membrane fragment transfer occurred within minutes (Fig. 3*B*). Furthermore, MHCII acquisition was completely abrogated by culturing NK cells and DCs in transwell plates (Fig. 3*C*), indicating that acquisition was dependent upon cell–cell contact. These results are consistent with the transfer of MHCII being mediated through trogocytosis rather than exosome secretion.

Trogocytosis is generally thought to be triggered by receptor signaling, and it has been reported that NK cells acquire MHCI and MHCI-related chain B from target cells by using NK cell receptors (NKRs) (21–23). Therefore, we conducted further studies to determine whether NKR signaling is required for MHCII acquisition. However, DAP10/12-deficient NK cells as well as WT NK cells acquired MHCII from DCs (Fig. S5*A*). WT NK cells also acquired MHCII from *β2-microglobulin*–deficient DCs that lacked cell surface expression of MHCI, indicating that MHCI-binding NKRs are not involved in this process (Fig. S5*A*). Consistent with these results, MHCII acquisition was not inhibited by an inhibitor of Syk family kinase, PI3K, or Src kinase (Fig. S5*B*). Neither did we observe the involvement of NK effector molecules such as perforin, IFN-γ, FasL, or TRAIL (24) in this process (Fig. S5). Interestingly, pretreatment of NK cells or DCs with cytochalasin D, an inhibitor of actin polymerization, or sodium azide, which depletes intracellular ATP, substantially inhibited MHCII transfer (Fig. S5*B*). Fixation of either NK cells or DCs with paraformaldehyde completely inhibited MHCII transfer (Fig. S5*B*). Collectively, these results suggest that activated NK cells acquire MHCII-containing DC membranes through a process that is dependent on plasma membrane and actin cytoskeleton interactions, but not NKR signaling or NK effector molecules.

MHCII-Dressed NK Cells Negatively Regulate $CD4^+$ T-Cell Proliferation. To explore whether MHCII-dressed NK cells could act as APCs to stimulate naïve T cells, we examined whether NK cells could also acquire costimulatory molecules from DCs by an in vitro coculture assay. Activated NK cells acquired CD80 and CD86, but the levels of these molecules on NK cells were minimal (Fig. S6).

We next examined whether MHCII-dressed NK cells could influence $CD4^+$ T-cell responses. We performed an antigen-presentation study using OT-II $CD4^+$ T cells specific for $OVA_{323–339}$/I-A^b. After coculture with WT or *H2-Ab1*–deficient

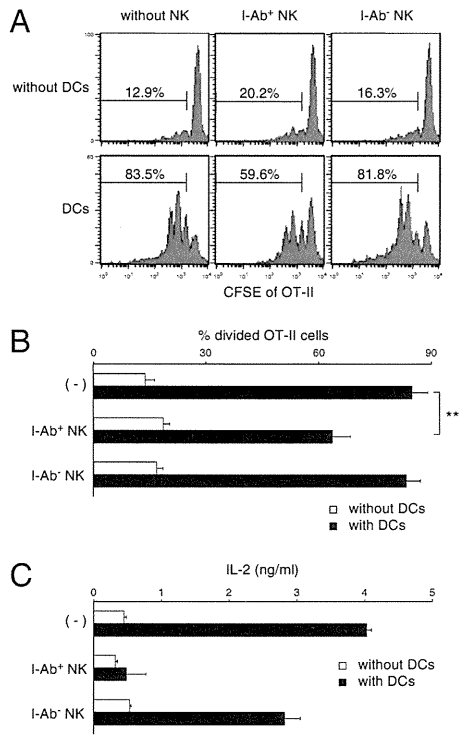

Fig. 4. $MHCII^+$ NK cells suppress $CD4^+$ T-cell proliferation induced by DCs. (*A*) NK cells were purified by cell sorting after coculture with WT DCs (I-A^{b+} NK) or *H2-Ab1*–deficient DCs (I-A^{b-} NK). CFSE-labeled OT-II $CD4^+$ T cells were cocultured with purified NK cells at a 1:10 ratio (NK:T), DCs at a 1:10 ratio (DC:T), or NK cells and DCs at a 1:1:10 ratio (NK:DC:T) in the presence of $OVA_{323–329}$ peptides (10 ng/mL) for 3 d. The CFSE intensity of OT-II $CD4^+$ cells was then analyzed. The percentages of divided OT-II cells are shown as means ± SD of triplicates in *B* (**$P < 0.01$ compared with DCs alone). (*C*) Production of IL-2 in the culture supernatants at 48 h after coculture was measured by ELISA. Similar results were obtained in three independent experiments.

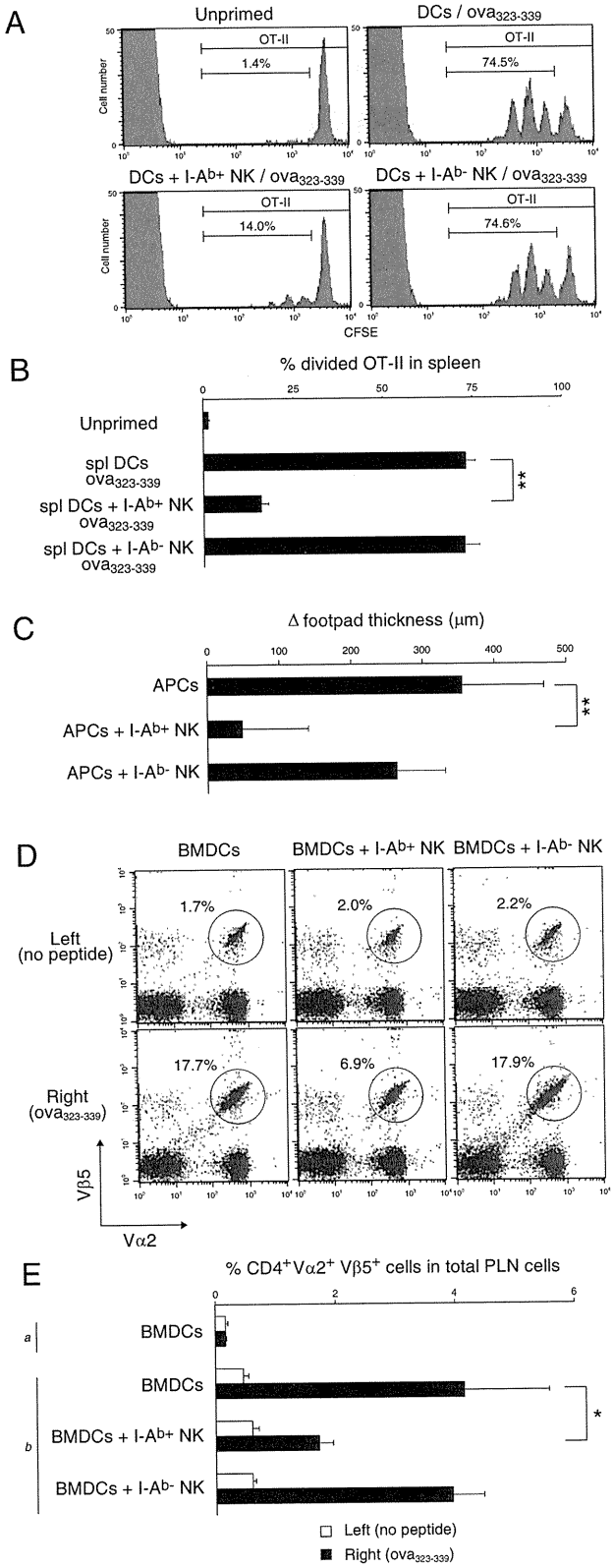

Fig. 5. MHCII$^+$ NK cells suppress CD4$^+$ T-cell responses in vivo. (*A*) B6 mice (n = 3) adoptively transferred with CFSE-labeled OT-II CD4$^+$ T cells were i.v. injected with $OVA_{323–339}$-loaded DCs or a mixture of these DCs and NK cells precultured with DCs together (I-A^{b+} NK cells) or separated (I-A^{b-} NK cells) as described in Fig. 3*C*. Two days later, CFSE intensity of CD4$^+$ Vα2$^+$ OT-II cells in spleen was analyzed. The percentage of divided OT-II cells is shown in *B*. B6 mice [n = 4 (*C*) or n = 3 (*D* and *E*)] adoptively transferred with activated OT-II CD4$^+$ T cells were s.c. injected with $OVA_{323–339}$-loaded APCs or a mixture of these APCs and I-A^{b+} NK or I-A^{b-} NK cells into the right footpads. The left footpads were injected with the same cell population without $OVA_{323–339}$ as controls. The following day, Δfootpad thickness was calculated by subtracting the left hind footpad thickness from the right (*C*). Three days after the s.c. injection, the percentage of Vα2$^+$ Vβ5$^+$ cells in total CD4$^+$ T cells in PLNs was analyzed (*D*) and the percentage of CD4$^+$ Vα2$^+$ Vβ5$^+$ cells in total PLN cells is calculated in *E*. (*a*) Naive mice. (*b*) OT-II CD4$^+$ T-cell transferred mice. *P < 0.05, **P < 0.01 compared with APCs alone. Similar results were obtained in two (*A* and *B*) or three (*C* and *D*) independent experiments. BMDCs, bone marrow-derived DCs.

DCs, I-A^b-dressed (I-A^{b+}) or I-A^b-negative (I-A^{b-}) NK cells were sort-purified. Purified I-A^{b+} NK cells did not induce naïve OT-II CD4$^+$ T-cell proliferation (Fig. 4 *A* and *B*), possibly due to insufficient expression of costimulatory molecules (Fig. S6). Interestingly, purified I-A^{b+} NK cells suppressed OT-II CD4$^+$ T-cell proliferation induced by DCs, whereas I-A^{b-} NK cells cocultured with *H2-Ab1*–deficient DCs did not (Fig. 4 *A* and *B*). Moreover, I-A^{b+} NK cells suppressed IL-2 production from OT-II CD4$^+$ T cells more effectively than I-A^{b-} NK cells (Fig. 4*C*). These results indicate that MHCII-dressed NK cells regulate CD4$^+$ T-cell responses to DCs via antigen presentation on MHCII without sufficient costimulation.

Furthermore, we addressed whether I-A^{b+} NK cells could suppress CD4$^+$ T-cell responses in vivo. Interestingly, I-A^{b+} NK cells significantly suppressed OT-II CD4$^+$ T-cell proliferation induced by DCs loaded with $OVA_{323–339}$ peptides in spleen, although I-A^{b-} NK cells did not affect the proliferation (Fig. 5 *A* and *B*). In a mouse model for delayed-type hypersensitivity (DTH) where activated OT-II CD4$^+$ T cells were i.v. transferred followed by s.c. injection with $OVA_{323–339}$-loaded APCs, I-A^{b+} NK cells attenuated footpad swelling (Fig. 5*C*) and reduced OT-II CD4$^+$ T-cell accumulation in the draining popliteal lymph nodes (PLNs) (Fig. 5 *D* and *E*). Taken together, these results suggest that MHCII-dressed NK cells suppress CD4$^+$ T-cell responses in vivo.

Discussion

In this study, we provide evidence that conventional murine NK cells do not transcriptionally induce MHCII but instead rapidly acquire MHCII protein from DCs through NK–DC interactions. These MHCII-dressed NK cells suppress CD4$^+$ T-cell responses to DCs by presenting antigen–MHCII complexes without sufficient costimulation, which might induce anergy in CD4$^+$ T cells. In addition, adoptive transfer of MHCII-dressed NK cells attenuated dermal DTH. Therefore, our findings may provide a mechanistic explanation for the negative immune regulation of T-cell immunity by NK cells.

Several recent studies have identified NK/DC hybrid-phenotype cells, which have functional properties characteristic of both NK cells and DCs (11, 25–28). IFN-producing killer DCs (IKDCs), also called B220$^+$ NK1.1$^+$ DCs, were identified as a novel DC subset harboring killer activity (27, 28). On the contrary, more recent studies have proposed that these killer DCs are functionally and developmentally activated NK cells (7–9). It remains unclear whether the MHCII-dressed NK cells we describe here are identical to the NK/DC hybrid-phenotype cells described in previous studies (25–28), although at least IKDCs and MHCII-dressed NK cells have similar antigenic phenotypes: CD11c$^+$ B220$^+$ MHCII$^+$ NKG2D$^+$ CD86^{dull+} Gr1$^-$ (Fig. S7). Interestingly, in a mouse model of type 1 diabetes, CD11c$^+$ DX5$^+$ cells, which are functionally and phenotypically similar to MHCII-dressed NK cells, were found to negatively regulate pathogenic T-cell activation (25). Of note, we observed that a small population of DCs cocultured with IL-2-activated NK cells became Ly49G2-positive (Fig. S8), suggesting that DCs

could also acquire NK cell surface proteins. Although this observation might also account for the generation of NK/DC hybrid-phenotype cells described in previous studies (25, 26), further studies will be necessary to characterize these hybrid-phenotype cells.

In addition, we observed that activated murine NK cells acquired MHCII from B cells in coculture experiments. However, the acquisition level of MHCII on these NK cells was lower than that on NK cells cocultured with DCs (Fig. S9), suggesting that activated NK cells preferentially acquired MHCII from DCs.

In contrast to mouse NK cells, after activation, all human NK cells synthesize HLA-DR as well as costimulatory molecules including CD80, CD86, and OX40 ligand (6, 11, 12). Unlike in humans, activation of mouse NK cells apparently does not induce the endogenous expression of MHCII.

Given that many cell types store a large excess of membrane on their cell surface (16), intercellular membrane transfer might occur frequently during immune cell–cell interactions. Recently, DCs have been reported to acquire peptide–MHCI complexes from distinct donor DCs and subsequently drive memory $CD8^+$ T-cell activation (19). T cells have also been reported to acquire CD80 and CD86 proteins from DCs by CTLA-4, thereby downmodulating the delivery of costimulatory signals (29). Our findings show that activated NK cells can acquire MHCII from DCs and regulate T-cell immune responses in vitro and in vivo. Taken together, it is possible that immune cells acquire additional functions and/or alter their intrinsic functions through intercellular transfer of immune regulatory molecules such as MHCII in lymphoid organs. Such newly generated cell populations could play important roles in the regulation of immune responses through their effects on other cell types.

Materials and Methods

Further details are available in *SI Materials and Methods*.

Mice. C57BL/6 mice were obtained from CLEA Japan. MHCII *H2-Ab1*–deficient mice (30) were kindly provided by D. Mathis (Harvard Medical School, Boston, MA). OT-II transgenic/rag-1 knockout mice were obtained from Taconic. These mice were maintained under specific pathogen-free conditions, and used according to the guidelines of the Institutional Animal Care and Use Committee established at Tohoku University.

RT-PCR. Total RNA was purified from cells using Sepasol (Nacalai). Complementary DNAs were synthesized from total RNAs by using oligo(dT) primer (Invitrogen). PCR was performed by using 10-fold serially diluted cDNA templates, AmpliTaq poly (Applied Biosystems), and primers listed in *SI Materials and Methods*.

NK–DC Interaction. Mouse splenic NK cells and DCs were prepared as described previously (31, 32). CFSE (0.5 μM)-labeled IL-2 (1,000 U/mL)-activated NK cells (5×10^5 per well) and splenic DCs (5×10^5 per well) were cocultured in a 96-well flat-bottom plate for the indicated periods at 37 °C. Then cells were stained with APC-labeled anti–I-A^b mAb (BioLegend) and analyzed on a FACSCanto II (BD Biosciences).

Confocal Microscopy. Cells were stained with 5- (and 6-) carboxytetramethylrhodamine succinimidyl ester (TAMRA; Invitrogen) or the following mAbs: AF488-anti–I-A^b, biotinylated anti-NK1.1 mAbs, and streptavidin-DyLight 594 (BioLegend). Then these cells were analyzed on a Carl Zeiss LSM510 confocal laser-scanning microscope equipped with a 63× objective lens as described previously (33).

In Vitro Antigen Presentation Assay. The antigen presentation assay was performed as described previously (32) with some modifications. Bone marrow-derived DCs (5×10^3 per well) and/or sort-purified NK cells (5×10^3 per well) were cocultured with CFSE (10 μM)-labeled OT-II $CD4^+$ T cells (5×10^4 per well) in a 96-well U-bottom plate for 3 d in the presence of $OVA_{323–339}$ peptides (10 ng/mL; Abgent). CFSE fluorescence intensity of OT-II $CD4^+$ T cells was analyzed by flow cytometry. Production of IL-2 in the culture supernatant at 48 h postaddition of OT-II $CD4^+$ T cells was measured by ELISA (eBioscience).

In Vivo OT-II Proliferation Assay. CFSE-labeled OT-II $CD4^+$ T cells (3×10^6 per mouse) were adoptively transferred into B6 mice. The following day, mice were i.v. injected with $OVA_{323–339}$ (1 μg/mL)-loaded splenic DCs (3×10^6 per mouse) or a mixture of these DCs (3×10^6 per mouse) and NK cells (3×10^6 per mouse). Two days later, mice were killed, and CFSE dilution of $CD4^+$ $V\alpha2^+$ splenocytes was analyzed by flow cytometry.

Dermal DTH. B6 mice adoptively transferred with activated OT-II $CD4^+$ T cells (6×10^6 per mouse) were s.c. injected with 50 μL of $OVA_{323–339}$-loaded APCs (2×10^7 per mouse) or a mixture of these APCs (2×10^7 per mouse) and NK cells (1×10^7 per mouse) into the right footpad. The left footpads were injected with the same cell population without $OVA_{323–339}$ as controls. Footpad thickness and infiltration of OT-II cells into PLNs were analyzed.

ACKNOWLEDGMENTS. We thank Hiromi Yoshida for cell sorting; Shota Endo and Hisaya Akiba for advice on OT-II experiments; and Chika Takahashi, Misato Tsugita, and Kazusa Ishizaki for technical assistance. This work was supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (to M.N., K.T., and K.O.); by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan H22-meneki-ippan-004 (to K.O.), and H22-meneki-ippan-005 (to M.N.); by a grant from the Takeda Science Foundation (to M.N.); and by a grant from the Mochia Memorial Foundation for Medical and Pharmaceutical Research (to M.N.).

1. Vivier E, et al. (2011) Innate or adaptive immunity? The example of natural killer cells. *Science* 331(6013):44–49.
2. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S (2008) Functions of natural killer cells. *Nat Immunol* 9:503–510.
3. Zitvogel L (2002) Dendritic and natural killer cells cooperate in the control/switch of innate immunity. *J Exp Med* 195(3):F9–F14.
4. Cooper MA, Fehniger TA, Fuchs A, Colonna M, Caligiuri MA (2004) NK cell and DC interactions. *Trends Immunol* 25(1):47–52.
5. Shi FD, Van Kaer L (2006) Reciprocal regulation between natural killer cells and autoreactive T cells. *Nat Rev Immunol* 6:751–760.
6. Spits H, Lanier LL (2007) Natural killer or dendritic: What's in a name? *Immunity* 26(1): 11–16.
7. Blasius AL, Barchet W, Cella M, Colonna M (2007) Development and function of murine $B220^+CD11c^+NK1.1^+$ cells identify them as a subset of NK cells. *J Exp Med* 204: 2561–2568.
8. Vosshenrich CA, et al. (2007) $CD11c^{lo}B220^+$ interferon-producing killer dendritic cells are activated natural killer cells. *J Exp Med* 204:2569–2578.
9. Caminschi I, et al. (2007) Putative IKDCs are functionally and developmentally similar to natural killer cells, but not to dendritic cells. *J Exp Med* 204:2579–2590.
10. Phillips JH, Le AM, Lanier LL (1984) Natural killer cells activated in a human mixed lymphocyte response culture identified by expression of Leu-11 and class II histocompatibility antigens. *J Exp Med* 159:993–1008.
11. Hanna J, et al. (2004) Novel APC-like properties of human NK cells directly regulate T cell activation. *J Clin Invest* 114:1612–1623.
12. Zingoni A, et al. (2004) Cross-talk between activated human NK cells and $CD4^+$ T cells via OX40-OX40 ligand interactions. *J Immunol* 173:3716–3724.
13. Roncarolo MG, et al. (1991) Natural killer cell clones can efficiently process and present protein antigens. *J Immunol* 147:781–787.
14. Mach B, Steimle V, Martinez-Soria E, Reith W (1996) Regulation of MHC class II genes: Lessons from a disease. *Annu Rev Immunol* 14:301–331.
15. Sokol CL, et al. (2009) Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 10:713–720.
16. Davis DM (2007) Intercellular transfer of cell-surface proteins is common and can affect many stages of an immune response. *Nat Rev Immunol* 7:238–243.
17. Joly E, Hudrisier D (2003) What is trogocytosis and what is its purpose? *Nat Immunol* 4:815.
18. Rechavi O, Goldstein I, Kloog Y (2009) Intercellular exchange of proteins: The immune cell habit of sharing. *FEBS Lett* 583:1792–1799.
19. Wakim LM, Bevan MJ (2011) Cross-dressed dendritic cells drive memory $CD8^+$ T-cell activation after viral infection. *Nature* 471:629–632.
20. Théry C, Zitvogel L, Amigorena S (2002) Exosomes: Composition, biogenesis and function. *Nat Rev Immunol* 2:569–579.
21. Roda-Navarro P, Vales-Gomez M, Chisholm SE, Reyburn HT (2006) Transfer of NKG2D and MICB at the cytotoxic NK cell immune synapse correlates with a reduction in NK cell cytotoxic function. *Proc Natl Acad Sci USA* 103:11258–11263.
22. Carlin LM, Eleme K, McCann FE, Davis DM (2001) Intercellular transfer and supramolecular organization of human leukocyte antigen C at inhibitory natural killer cell immune synapses. *J Exp Med* 194:1507–1517.

23. Sjöström A, et al. (2001) Acquisition of external major histocompatibility complex class I molecules by natural killer cells expressing inhibitory Ly49 receptors. *J Exp Med* 194:1519–1530.
24. Smyth MJ, Hayakawa Y, Takeda K, Yagita H (2002) New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat Rev Cancer* 2:850–861.
25. Homann D, et al. (2002) CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. *Immunity* 16:403–415.
26. Pillarisetty VG, Katz SC, Bleier JI, Shah AB, Dematteo RP (2005) Natural killer dendritic cells have both antigen presenting and lytic function and in response to CpG produce IFN-γ via autocrine IL-12. *J Immunol* 174:2612–2618.
27. Taieb J, et al. (2006) A novel dendritic cell subset involved in tumor immunosurveillance. *Nat Med* 12:214–219.
28. Chan CW, et al. (2006) Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity. *Nat Med* 12:207–213.
29. Qureshi OS, et al. (2011) Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332:600–603.
30. Cosgrove D, et al. (1991) Mice lacking MHC class II molecules. *Cell* 66:1051–1066.
31. Ogasawara K, et al. (2003) Impairment of NK cell function by NKG2D modulation in NOD mice. *Immunity* 18(1):41–51.
32. Nakayama M, et al. (2009) Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. *Blood* 113:3821–3830.
33. Nakayama M, et al. (2007) Paired Ig-like receptors bind to bacteria and shape TLR-mediated cytokine production. *J Immunol* 178:4250–4259.

Supporting Information

Nakayama et al. 10.1073/pnas.1110584108

SI Materials and Methods

Mice. C57BL/6 mice were obtained from CLEA Japan. MHCII *H2-Ab1*–deficient mice (1) were kindly provided by D. Mathis (Harvard Medical School, Boston, MA). C57BL/6 mice deficient in *perforin*, *IFN-γ*, or *β2-microglobulin* were obtained from The Jackson Laboratory. OT-II transgenic/rag-1 knockout mice were obtained from Taconic. DAP12 knockout mice and DAP10/12 double knockout mice were generated as described previously (2).

Adoptive Natural Killer Cell Transfer Assay. Natural killer (NK) cells were prepared from WT B6 mice or *H2-Ab1* knockout mice as described previously (3). In brief, spleen cells were incubated with anti-CD4 and anti-CD8 mAbs, followed by magnetic beads coated with goat anti-mouse Ig and goat anti-rat Ig Abs (Advanced Magnetics). After removing CD4, CD8, and surface Ig-positive cells by magnetic cell sorting, NK cells were stained with phycoerythrin (PE)-labeled DX5 mAb (BioLegend) and purified using anti-PE MACS beads (Miltenyi Biotec). Purified NK cells were cultured with rhIL-2 (1,000 U/mL; Wako) for 5 d. These activated NK cells (5×10^6 per mouse) were labeled with 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE) (2 μM; Invitrogen) and transferred i.v. into WT B6 mice or *H2-Ab1* knockout mice. The following day, mice were killed, and splenocytes were stained with PE–anti-NK1.1 and antigen-presenting cell (APC)–anti–I-A^b mAbs (BioLegend). Expression of I-A^b and NK1.1 on $CFSE^+$ cells was analyzed on a FACSCanto II (BD Biosciences).

RT-PCR. Splenic $NK1.1^+$ cells were purified from polyI:C (100 μg per mouse; InvivoGen)-treated mice or naive mice as described above. Splenic $CD11c^+$ dendritic cells (DCs) were purified as described previously (4). In brief, spleens were digested with 400 U/mL collagenase (Wako) in the presence of 5 mM EDTA and separated into low- and high-density fractions on an OptiPrep gradient (Axis-Shield). Low-density cells were purified using anti-CD11c MACS beads (Miltenyi Biotec). Then $I\text{-}Ab^+$ $NK1.1^+$ cells or $NK1.1^-$ $CD11c^+$ cells were further purified on an Epics ALTRA cell sorter (Beckman Coulter) (>98% $I\text{-}Ab^+$ $NK1.1^+$ cells; >96% $NK1.1^-$ $CD11c^+$ cells). Total RNA was purified from these purified cells using Sepasol (Nacalai). Complementary DNAs were synthesized from total RNAs by using oligo(dT) primer (Invitrogen). PCR was performed by using 10- or 100-fold serially diluted cDNA templates and AmpliTaq poly (Applied Biosystems). Primers used in this study were as follows: Klrk1-F, 5′-ATG GCA TTG ATT CGT GAT CGA AAG-3′; Klrk1-R, 5′-TTA CAC CGC CCT TTT CAT GCA GAT-3′; H2-Ab1-F, 5′-CGC ATA CGA TAT GTG ACC AGA TAC-3′; H2-Ab1-R, 5′-CAA CAT CAT TTT GCT CCA GGC AGA CTC-3′; class II transactivator (Ciita)-F, 5′-ATG CGC TGC CTG GTT CCT GGC CCT TCT-3′; Ciita-R, 5′-TCC CTG GGG CAG AGT GGT GAA GAT-3′; Actb-F, 5′-ATG GAT GAC GAT ATC GCT GCG CTG-3′; Actb-R, 5′-GTA GCC ACG CTC GGT CAG GAT CTT-3′.

ELISA for DC-Derived Exosomes. Anti-mouse CD9 mAb (10 μg/mL; BioLegend) was precoated on a 96-well EIA/RIA plate (Costar). After blocking with 5% FCS/PBS at room temperature for 1 h, the culture supernatants were added to the wells and incubated at room temperature for 2 h. After washing with PBS containing 0.05% Tween 20, exosomes were detected by biotinylated anti–I-A^b mAb (BioLegend) and streptavidin-HRP (eBioscience).

NK–DC Interaction. CFSE (0.5 μM)-labeled IL-2 (1,000 U/mL)-activated NK cells (5×10^5 per well) and splenic DCs (5×10^5 per well) were cocultured in a 96-well flat-bottom plate for the indicated periods at 37 °C. For the transwell assay, NK cells (3×10^6 per well) were cultured in the upper chamber of a 12-well plate and separated from DCs (3×10^6 per well) by a 0.4 μm-pore semipermeable membrane (Greiner Bio-One). As controls, NK cells and DCs (3×10^6 per well) were cultured together in a 12-well plate. For the inhibition assay, NK cells or DCs were pretreated for 1 h with the following reagents: piceatannol (100 μM), LY294002 (100 μM), cytochalasin D (20 μM), PP2 (50 μM), paraformaldehyde (4%; Wako), polyI (10 μg/mL), dextran sulfate (10 μg/mL; Sigma), RGDS peptides (10 mM; Peptide Institute), NaN_3 (50 mM; Nacalai), and mAbs against FcγRIIb/III (2.4G2, 10 μg/mL; BD Biosciences), FasL (K10, 10 μg/mL), or TRAIL (N2B2, 10 μg/mL; BioLegend). After coculture for the indicated times, cells pretreated with 2.4G2 mAb were stained with the following mAbs: APC-labeled anti–I-A^b, anti-CD80, anti-CD86, or anti-CD11c (BioLegend). In some assays, NK cells were then purified by cell sorting and analyzed via flow cytometry.

In Vitro Antigen Presentation Assay. The antigen presentation assay was performed as described previously (4) with some modifications. Bone marrow-derived DCs (BMDCs) were generated by rmGM-CSF (40 ng/mL; PeproTech). CFSE-labeled NK cells (1×10^7 per well) and BMDCs (5×10^6 per well) were cocultured in a 6-well plate for 1 h. Then these cells were stained with APC-anti–I-A^b mAb, followed by sorting into $I\text{-}A^{b+}$ and $I\text{-}A^{b-}$ NK subsets on an Epics ALTRA cell sorter (Beckman Coulter) (>98% $I\text{-}A^{b+}$ $CFSE^+$; >99% $I\text{-}A^{b-}$ $CFSE^+$). BMDCs (5×10^3 per well), and/or sorted NK cells (5×10^3 per well) were cocultured with CFSE (10 μM)-labeled OT-II $CD4^+$ T cells (5×10^4 per well) in a 96-well U-bottom plate for 3 d in the presence of $OVA_{323–339}$ peptides (10 ng/mL; Abgent). CFSE fluorescence intensity of OT-II $CD4^+$ T cells was analyzed by flow cytometry. Production of IL-2 in the culture supernatant at 48 h post-addition of OT-II $CD4^+$ T cells was measured by ELISA (eBioscience). For statistical evaluation, an unpaired Student's *t* test, two-tailed, was used. *P* values less than 0.05 were considered significant.

In Vivo OT-II $CD4^+$ Cell Proliferation Assay. OT-II $CD4^+$ cell proliferation in spleen was analyzed as described previously (4) with some modifications. CFSE-labeled naive OT-II $CD4^+$ T cells (3×10^6 per mouse) were adoptively transferred into B6 mice. The next day, mice were i.v. injected with $OVA_{323–339}$ (1 μg/mL)-loaded splenic DCs (3×10^6 per mouse) or a mixture of these DCs (3×10^6 per mouse) and NK cells cocultured with these DCs together ($I\text{-}A^{b+}$ NK cells; 3×10^6 per mouse) or separately ($I\text{-}A^{b-}$ NK cells; 3×10^6 per mouse). Two days later, mice were killed, and CFSE dilution of $CD4^+$ $V\alpha2^+$ splenocytes was analyzed by flow cytometry. For statistical evaluation, an unpaired Student's *t* test, two-tailed, was used. *P* values less than 0.05 were considered significant.

Dermal Delayed-Type Hypersensitivity. A dermal delayed-type hypersensitivity study was performed as described previously (5) with some modifications. OT-II $CD4^+$ T cells were stimulated with $OVA_{323–339}$ (100 ng/mL) for 3 d, and then cells were maintained in IL-2 (10 U/mL)-containing medium without $OVA_{323–339}$ for another 3 d. These cells (6×10^6 per mouse) were transferred i.v. into B6 mice. The next day, recipient mice

were s.c. injected with 50 μL of $OVA_{323–339}$-loaded APCs (collagenase-digested whole splenocytes or BMDCs; 2×10^7 each per mouse) or a mixture of these APCs (2×10^7 per mouse) and NK cells cocultured with these DCs together ($I\text{-}A^{b+}$ NK cells; 1×10^7 per mouse) or separately ($I\text{-}A^{b-}$ NK cells; 1×10^7 per mouse) into the right footpad. The left footpads were injected with the same cell population without $OVA_{323–339}$ as controls. The following day, Δfootpad thickness was calculated by subtracting the left hind footpad thickness from the right. Three days after the s.c. injection, the OT-II $CD4^+$ $V\alpha2^+$ $V\beta5^+$ cell population migrating into popliteal lymph nodes was measured by flow cytometry. For statistical evaluation, an unpaired Student's *t* test, two-tailed, was used. *P* values less than 0.05 were considered significant.

1. Cosgrove D, et al. (1991) Mice lacking MHC class II molecules. *Cell* 66:1051–1066.
2. Inui M, et al. (2009) Signal adaptor DAP10 associates with MDL-1 and triggers osteoclastogenesis in cooperation with DAP12. *Proc Natl Acad Sci USA* 106:4816–4821.
3. Ogasawara K, et al. (2003) Impairment of NK cell function by NKG2D modulation in NOD mice. *Immunity* 18(1):41–51.
4. Nakayama M, et al. (2009) Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. *Blood* 113:3821–3830.
5. Ruth AJ, et al. (2004) An IL-12-independent role for CD40-CD154 in mediating effector responses: Studies in cell-mediated glomerulonephritis and dermal delayed-type hypersensitivity. *J Immunol* 173(1):136–144.

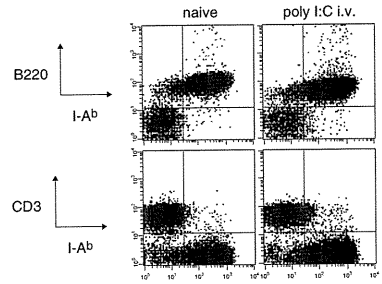

Fig. S1. Expression of MHCII on B and T cells. C57BL/6 mice were i.v. injected with 100 μg of polyI:C. The following day, collagenase-digested splenocytes from these mice or naive C57BL/6 mice were stained with anti-CD3, anti-B220, and anti–$I\text{-}A^b$ mAbs. Similar results were obtained in three independent experiments.

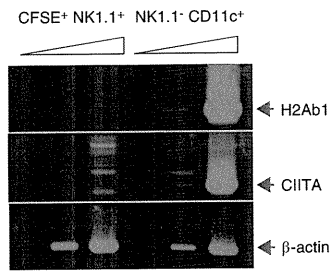

Fig. S2. Expression of H2-Ab1 and CIITA transcripts in purified $CFSE^+$ $I\text{-}Ab^+$ $NK1.1^+$ cells or $NK1.1^-$ $CD11c^+$ cells. IL-2-activated CFSE-labeled NK cells (5×10^6 per mouse) were transferred into mice. The following day, $CFSE^+$ $NK1.1^+$ cells in spleen were purified by cell sorting. $CD11c^+$ cells were purified as described in *SI Materials and Methods*. Expression of the indicated transcripts in purified cells was analyzed by semiquantitative RT-PCR using 100-fold serially diluted cDNA templates. Similar results were obtained in two independent experiments.

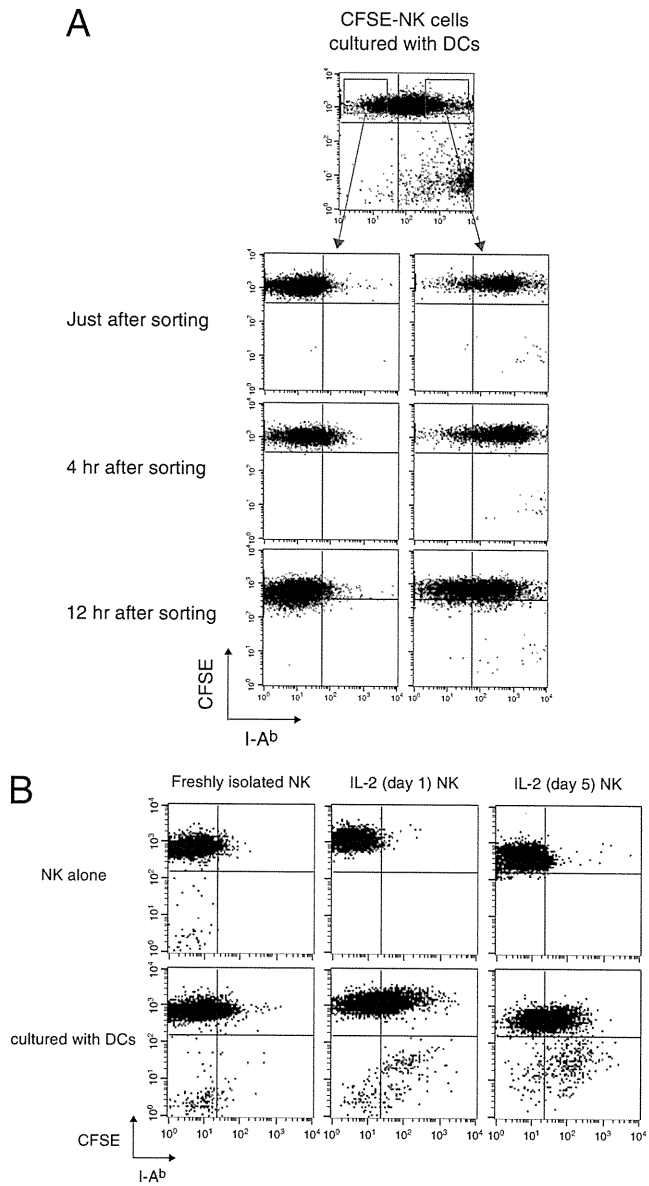

Fig. S3. Activated NK cells acquire MHCII. (*A*) Stability of MHCII on NK cells. I-Ab^{-} and I-Ab^{+} NK cells were purified by cell sorting after coculture with WT DCs. After the indicated times, cells were stained with anti–I-A^{b} mAb. Similar results were obtained in three independent experiments. (*B*) Freshly isolated NK cells do not acquire MHCII. Freshly isolated splenic NK cells or IL-2 (1,000 U/mL)-activated NK cells were labeled with CFSE and then cocultured with splenic CD11c^{+} cells at a 1:1 ratio for 1 h. I-A^{b} expression on NK1.1^{+} cells was analyzed. Similar results were obtained in two independent experiments.

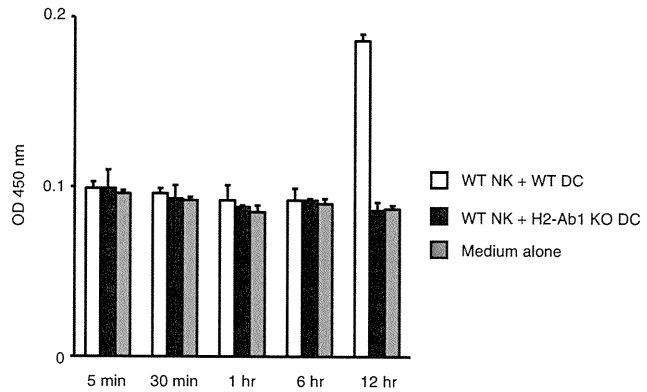

Fig. S4. Exosomes in NK/DC coculture supernatants. IL-2-activated WT NK cells were cocultured with WT or H2-Ab1 knockout splenic DCs at a 1:1 ratio for the indicated times. Exosomes in culture supernatants were detected by sandwich ELISA. Similar results were obtained in two independent experiments.

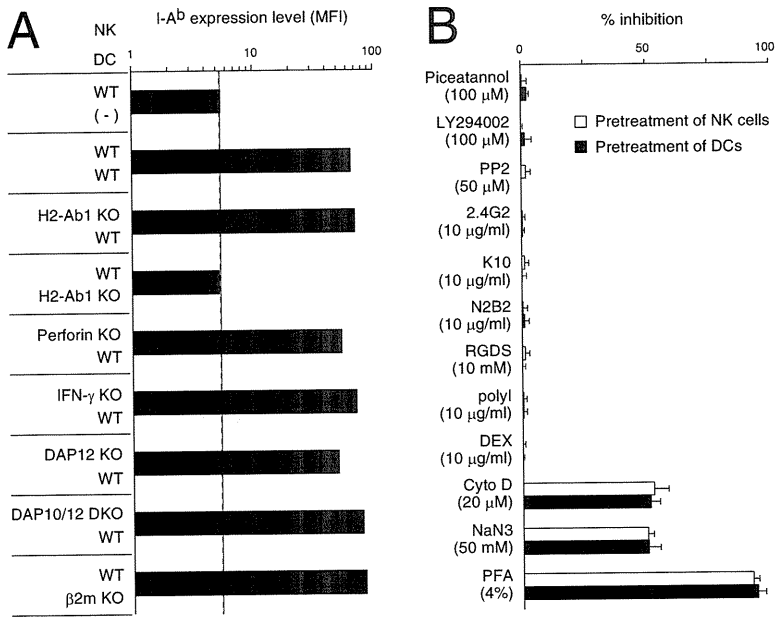

Fig. S5. Intercellular MHCII transfer is NK cell receptor-independent. (*A*) CFSE-labeled NK cells and splenic DCs from the indicated mice were cocultured for 30 min. Surface expression of I-A^b on NK cells was analyzed by flow cytometry, and the mean fluorescence intensity (MFI) is indicated. (*B*) NK cells (white columns) or DCs (black columns) were pretreated with the following inhibitors 1 h before coculture: piceatannol (a Syk family kinase inhibitor), LY294002 (a specific inhibitor of PI3K), PP2 (a Src kinase inhibitor), 2.4G2 (an anti-FcγRIIb/III–blocking mAb), K10 (an anti-FasL–blocking mAb), N2B2 (an anti-TRAIL–blocking mAb), RGDS (an integrin-antagonizing peptide), polyI (polyinosinic acid; a negatively charged reagent), DEX (dextran sulfate; a negatively charged reagent), cytochalasin D (an inhibitor of actin polymerization), NaN_3 (an ATP-depleting reagent), and PFA (paraformaldehyde; a cross-linking reagent). The expression level of I-A^b on NK cells was analyzed by flow cytometry. Percent inhibition was calculated by comparing the MFI in the presence of an inhibitor with the MFI in the absence of inhibitor. Data represent means + SD of triplicates. Similar results were obtained in three independent experiments.

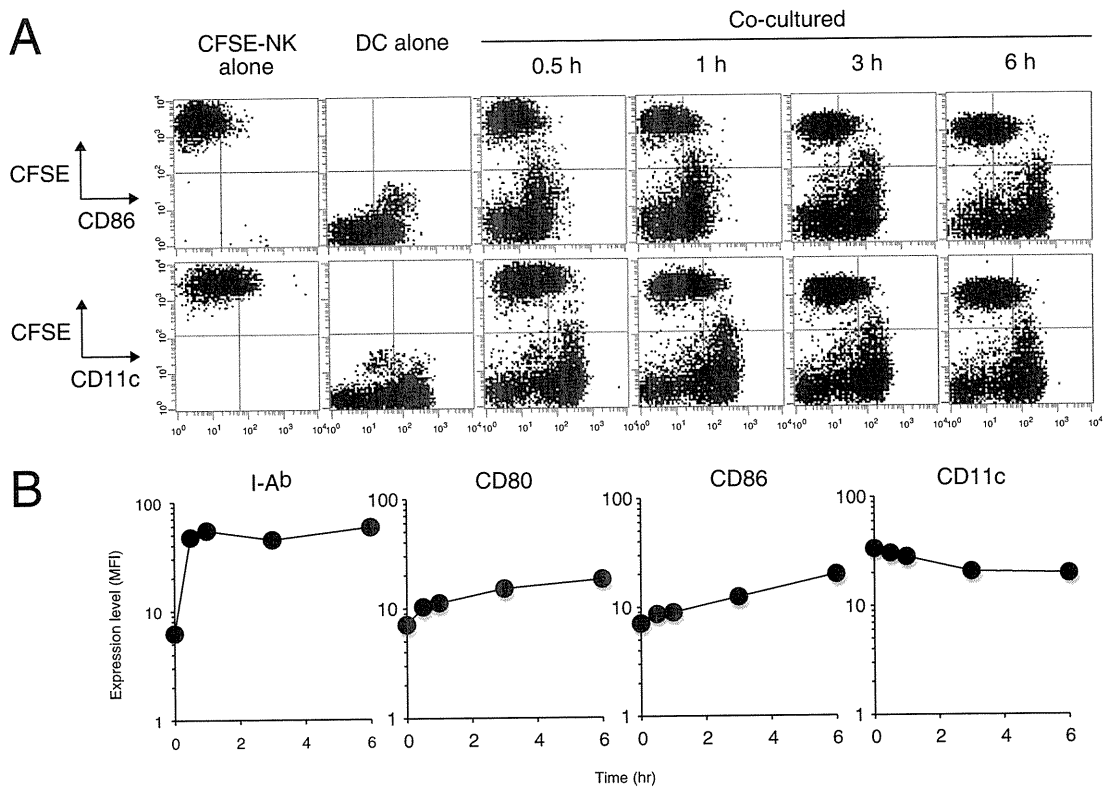

Fig. S6. Kinetic analysis of intercellular transfer of MHCII, CD80, CD86, and CD11c. (*A*) CFSE-labeled NK cells were cocultured with DCs at a 1:1 ratio. After the indicated periods, cells were stained with anti-CD86 and anti-CD11c mAbs. (*B*) The MFI of I-A^b, CD80, CD86, and CD11c expression on NK cells cocultured with DCs was analyzed by flow cytometry. Data represent the mean of triplicates. Similar results were obtained in two independent experiments.

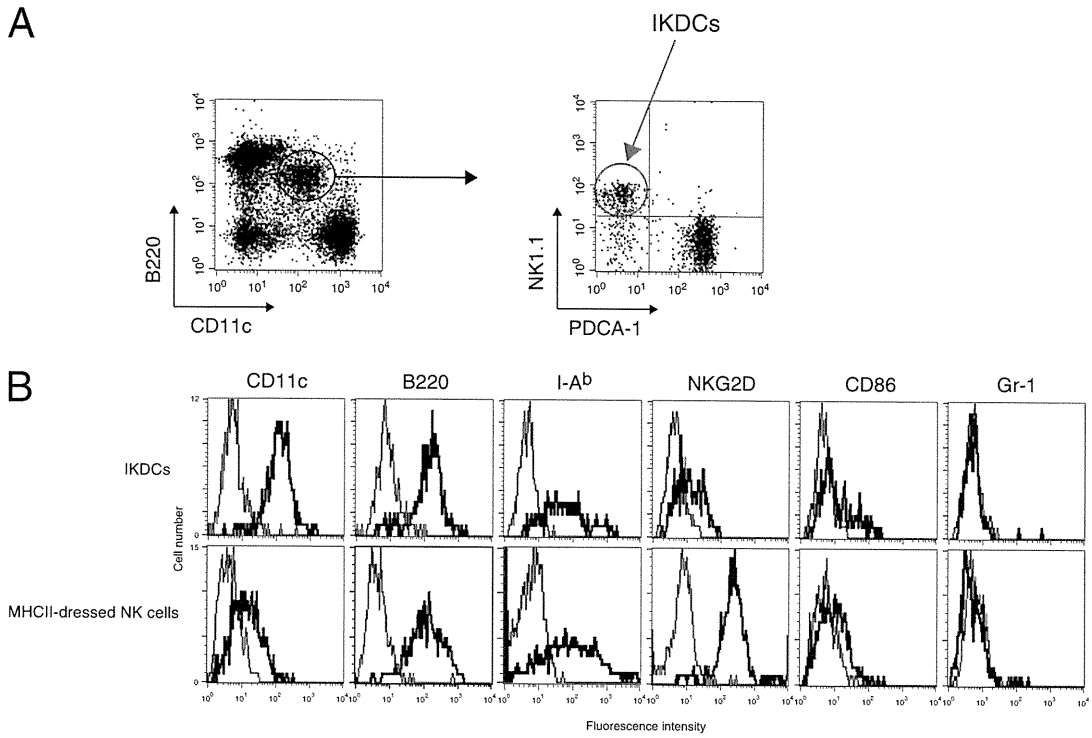

Fig. S7. Antigenic phenotypes of IFN-producing killer DCs (IKDCs) and MHCII-dressed NK cells. (*A*) Collagenase-digested low-density spleen cells were stained with anti-B220, anti-CD11c, anti-NK1.1, and anti–PDCA-1 mAbs. The putative IKDC population is indicated by a red arrow. (*B*) Putative IKDCs and MHCII-dressed NK cells were stained with the indicated mAb (thick histograms) or respective isotype control mAb (thin histograms). Similar results were obtained in three independent experiments.

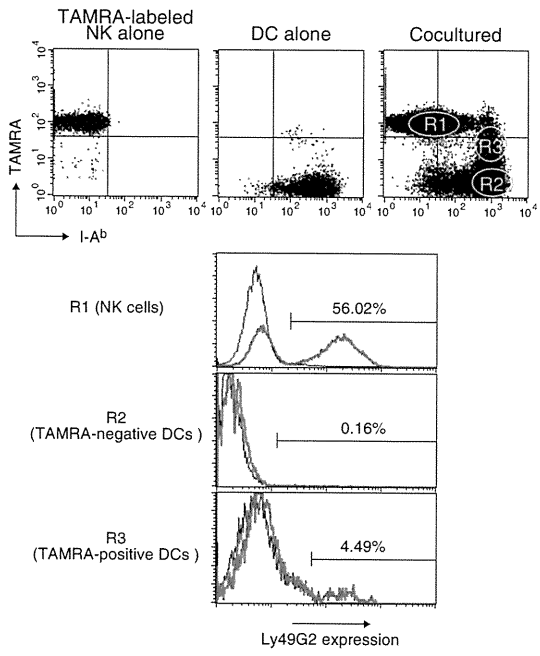

Fig. S8. DC acquisition of Ly49G2 from NK cells. TAMRA [5- (and 6-) carboxytetramethylrhodamine succinimidyl ester]-labeled activated NK cells were co-cultured with splenic DCs at a 1:1 ratio for 1 h. Then cells were stained with anti–I-A^b and anti-Ly49G2 mAbs or control rat IgG_{2a}. In histograms, black and red lines indicate control $rIgG_{2a}$ and anti-Ly49G2 mAb staining, respectively. The percentage of Ly49G2-positive cells in R1, R2, and R3 is indicated. Similar results were obtained in two independent experiments.

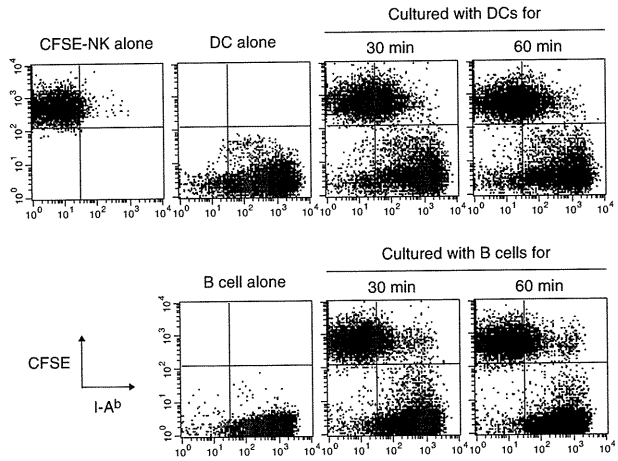

Fig. S9. NK acquisition of MHCII from B cells. CFSE-labeled activated NK cells were cocultured with splenic DCs or B cells at a 1:1 ratio for the indicated periods of time. Then cells were stained with anti–I-A^b mAb. Similar results were obtained in two independent experiments.

THE JOURNAL OF IMMUNOLOGY

High Clonality of Virus-Specific T Lymphocytes Defined by TCR Usage in the Brains of Mice Infected with West Nile Virus

Kazutaka Kitaura, Yoshiki Fujii, Daisuke Hayasaka, Takaji Matsutani, Kenji Shirai, Noriyo Nagata, Chang-Kweng Lim, Satsuki Suzuki, Tomohiko Takasaki, Ryuji Suzuki and Ichiro Kurane

J Immunol; Prepublished online 9 September 2011;
doi:10.4049/jimmunol.1100442
http://www.jimmunol.org/content/early/2011/09/09/jimmunol.1100442

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.