THE FUNCTION OF Ia$^+$ DENDRITIC CELLS AND Ia$^-$ DENDRITIC CELL PRECURSORS IN THYMOCYTE MITOGENESIS TO LECTIN AND LECTIN PLUS INTERLEUKIN 1

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When labeled nucleosides ([^{3}H]thymidine, bromodeoxyuridine) are administered to rodents, a large fraction of thymocytes are noted to synthesize DNA (1–6). Extensive cell proliferation may be critical for the production of peripheral T lymphocytes with an appropriately diverse repertoire of specificities. The repertoire also appears to be shaped within the thymus. Clones that react with self MHC (self tolerance) are deleted (7), while clones that respond to antigen plus self MHC (self MHC restriction) are selected (8, 9).

Little is known about the types of accessory cells, particularly thymic accessory cells, that are required for thymocyte proliferation. Thymic dendritic cells have been identified in birds (10), mice (11), rats (12, 13), and man (14). We have been interested in evaluating the effects of dendritic cells on thymus function in vitro given the important accessory role of this cell type in the growth and stable binding of MHC-restricted T cells (15, 16). In addition, IL-1 acts as a cofactor for thymocyte proliferation (17). Recent experiments have shown that the enhancing effect of IL-1 on peripheral T cell growth is exerted primarily at the level of dendritic cells (18).

Here we describe methods for enriching and depleting the trace population of dendritic cells that is present in suspensions of teased mouse thymi. We use this information to show that dendritic cells are powerful accessory cells for thymocyte mitogenesis. IL-1 not only enhances dendritic function 3–10-fold; it also has the unusual property of inducing the formation of typical dendritic cells from Ia$^-$ precursors.

Materials and Methods

Mice. Both sexes were used at 5–8 wk. C57BL/6 × DBA/2 F1, BALB/c × DBA/2, and B6.H-2k mice were from Trudeau Institute, Saranac Lake, NY. C × D2 F1 mice were from Shizuota Agricultural Corp., Shizuoka, Japan.

IL-1 and Anti-IL-1. Murine rIL-1α (5×10^6 U/mg) was a gift from Dr. P. LoMedico, Hoffman-Laroche, Nutley, NJ (19). Human rIL-1α ($2–5 \times 10^6$ U/mg) was provided by

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Dr. LoMedico and by Dainippon Pharmaceutical, Osaka, Japan (9). The Ig fraction of anti-human IL-1α (20), and a murine mAb to human IL-1α/β (unpublished data), were generously supplied by Drs. R. Newton (DuPont, Glenolden, PA) and Dainippon Co., respectively.

Culture Medium. We used RPMI 1640 supplemented with 5% FCS, 5×10^{-5} M 2-ME, and 20 μg/ml gentamicin.

Thymocytes. Thymus suspensions were prepared by teasing with forceps followed by further disruption on stainless steel sieves in RPMI 1640 medium. Ia^+ cells were depleted by treatment with anti-Ia mAb (either B21-2, anti-I-A; 14-4-5S anti-I-E; American Type Culture Collection, Rockville, MD) (21, 22) and rabbit complement (Pel-Freeze Biologicals, Rodgers, AR; Cedarlane Laboratories Ltd., Hornsby, Ontario, Canada). To verify the depletion of Ia^+ cells with anti-I-A, we stained with biotin–anti-I-E followed by PE-avidin (Becton Dickinson & Co., Mountain View, CA); alternatively we used FITC-B21-2 to monitor depletion with anti-I-E and complement (23). To further deplete accessory cells, including Ia^- dendritic cell precursors described in Results, thymus suspensions were passed over nylon wool. $1–2 \times 10^9$ cells in 3–4 ml of medium were applied to 3-g nylon-wool columns at 37°C and eluted 1 h later with medium supplemented with 20 mM Hepes at pH 7.2. Treatment with anti-Ia and complement, or passage over nylon wool, each removed ~25% of total thymocytes.

Accessory Cells. Splenic dendritic cells were enriched from low-density spleen adherent fractions (24). Macrophages were resident peritoneal cells selected by adherence to plastic for 6 h. Thymic dendritic cells accounted for 0.1% of the cells from manually teased thymi (Results). To enrich for these cells, suspensions were separated into low- and high-density fractions on dense Percoll columns. $15–25 \times 10^7$ thymus cells were suspended in 1.5 ml medium and applied to 5 ml of 58.5% isotonic Percoll (Pharmacia Fine Chemicals, Piscataway, NJ; 6.5 parts isoperc, 1 part FCS, and 2.5 parts PBS). The low-density fraction contained 40–50% of the thymocytes and all of the dendritic cells, but was depleted of red and dead cells. Most thymocytes were depleted with mAb GK 1.5 anti-CD4 (25) and HO 2.2 anti-CD8 (26) (American Type Culture Collection), and most B cells with rabbit anti–mouse Ig and complement. Dendritic cell frequencies were determined with FITC-B21-2 anti-Ia mAb followed by flow cytometry or microscopy (1 μg/ml; Fig. 3). These Ia^+,Ig^- cells were considered to be dendritic cells because most were Fc receptor–negative and 33D1 antigen-positive, and because macrophages were lacking using staining with mAb to M1/70 and F4/80 antigens (27).

Mitogenesis Assays. 3×10^5 thymocytes were cultured in 0.2 ml medium in 6-mm flat-bottomed microtest wells with graded doses of accessory cells and Con A (1 μg/ml; Sigma Chemical Co., St. Louis, MO, or Pharmacia Fine Chemicals). Mitogenesis was measured at 48–60 h by adding [^{3}H]TdR at 1 μCi/well in 50 μl medium. All data are means of triplicates in which the standard deviations were <10% of the mean. In some experiments, we tested the stimulating activity of thymic dendritic cells for allogeneic peripheral T lymphocytes. The culture conditions were the same as above, except that the T cells were nylon wool–nonadherent, Ia^- spleen and proliferation was measured at 96–108 h.

Results

Accessory Cell Dependence of Thymocyte Mitogenesis to Con A and IL-1. Murine thymus suspensions reliably showed some proliferative activity when challenged with 1 μg/ml Con A in culture, and this proliferation was enhanced by exogenous murine or human IL-1α. The fact that thymocytes responded to Con A implied that some endogenous accessory cells were present. A combination of two methods was required to prepare thymocytes that were unresponsive to Con A unless supplemented with *exogenous* accessory cells: treatment with anti-Ia and complement, and passage over nylon wool (left hand column of data in Table I).

Graded doses of dendritic cells progressively enhanced the thymocyte responses to lectin, and lectin plus IL-1 (Table I, Fig. 1). 0.1–0.3% spleen dendritic

TABLE I

Accessory Cell Requirements for Thymocyte Mitogenesis to IL-1

Responding thymocytes (3×10^5)	rIL-1α added (50 U/ml)	Proliferative response to Con A + graded doses of dendritic cells				
		None	300	1,000	3,000	10,000
		cpm $\times 10^{-3}$				
Unfractionated	None	12.5	21.2	36.2	86.0	121.8
	Murine	37.6	49.8	84.2	147.9	174.9
	Human	33.8	50.6	77.5	136.5	184.2
Ia$^-$	None	5.8	14.7	38.7	103.9	206.7
	Murine	19.7	35.9	100.9	220.2	283.4
	Human	19.0	37.4	105.9	204.0	281.0
Nylon nonadherent	None	2.6	11.6	35.2	121.0	174.7
	Murine	7.1	33.6	98.6	222.6	264.9
	Human	6.7	32.3	86.2	222.7	269.6
Ia$^-$ nylon nonadherent	None	0.4	4.1	15.0	53.9	107.6
	Murine	0.7	12.6	39.7	109.2	147.1
	Human	0.7	11.1	45.4	128.6	166.0

Different populations of thymus cells (left column) were stimulated with 1 μg/ml Con A, murine and human rIL-1α, and graded doses of dendritic cells. Replicate cultures were stimulated in the absence of Con A but the proliferative responses were <1,000 cpm and are not shown. Note that treatment with anti-Ia and complement, followed by passage over nylon wool, ablates the "background" mitogenesis response.

cells restored mitogenic activity to that seen in unfractionated thymus. Higher dendritic doses yielded higher responses. Adherent peritoneal cells, primarily macrophages, were 30 times less active than dendritic cells in supporting thymocyte proliferation as reported elsewhere (28), but the function of macrophages was *not* enhanced by either murine or human IL-1α (Fig. 1).

Evidence that IL-1 Can Act at the Level of Dendritic Cells. Splenic dendritic cells, which were >90% pure (24), were cultured with IL-1, washed, and then added to thymocytes in the presence or absence of lectin and/or additional IL-1. IL-1-pulsed dendritic cells were 3–10-fold more active than untreated dendritic cells (Fig. 2, compare Δ and $\bigcirc$) Pretreating the dendritic cells beforehand with IL-1 was as effective as IL-1 added continuously to the mitogenesis assay (compare Δ and $\bullet$). However, IL-1 did not amplify mitogenesis when it was used to pretreat the responding thymocytes (compare $\square$ and $\bigcirc$). The amplifying effect of IL-1 was comparable over a wide dose range (1–50 U/ml).

To pursue the dendritic cell as a site of action of IL-1, we tested the effect of neutralizing antibodies. Anti-IL-1 totally blocked the amplifying effect of IL-1 when the cytokine was added continuously to the thymocyte assay (Table II, compare groups 3 and 4), but the antibody did not inhibit the IL-1-independent response to Con A (compare groups 1 and 2). The amplified function of IL-1-pretreated dendritic cells (compare groups 1 and 5) was resistant to anti-IL-1 (compare groups 5 and 6). These results indicate that IL-1 is not carried over into the thymocyte assay when used to pretreat dendritic cells. Instead, cytokine

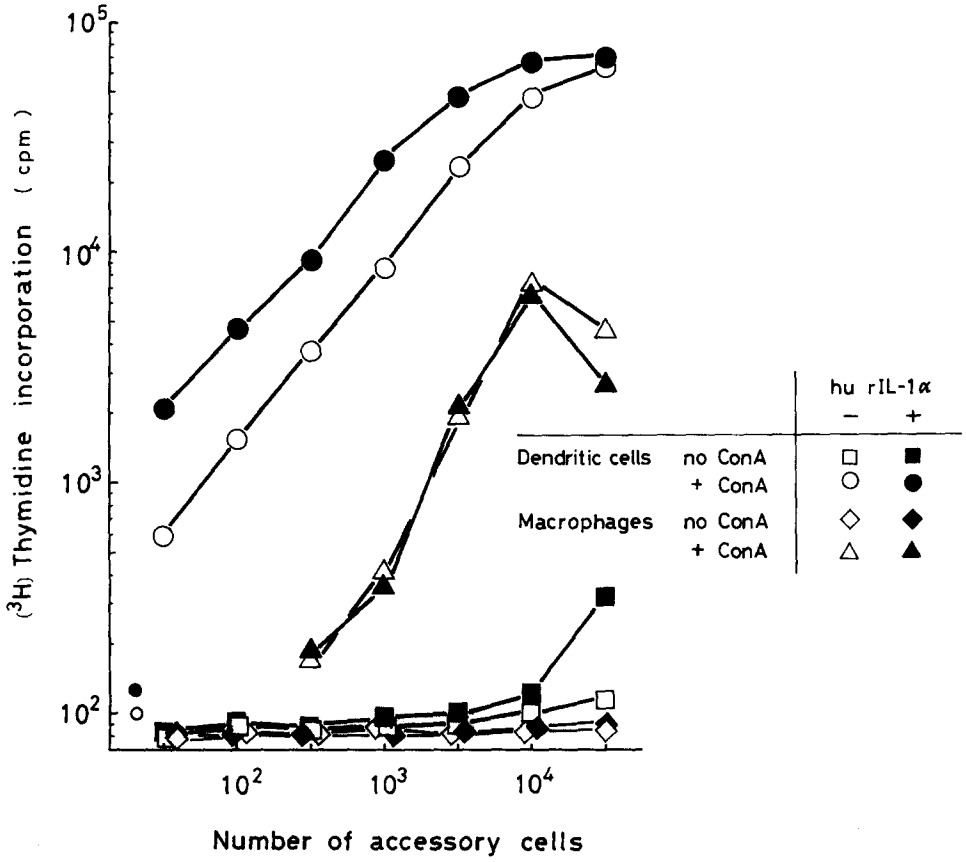

FIGURE 1. Efficacy of dendritic cells and macrophages in thymocyte responses to Con A and Con A plus IL-1. Nylon wool–nonadherent, Ia$^-$ thymocytes were challenged with Con A, IL-1, and graded doses of accessory cells as indicated. Note that dendritic cells are 30 times more potent than macrophages (compare circles and triangles), and that their function can be enhanced by exogenous rIL-1α (here human IL-1 at 50 U/ml; compare open and closed symbols).

alters the dendritic cell so that it is a better accessory cell for thymocyte mitogenesis.

Identification and Function of Dendritic Cells in Thymocyte Suspensions. In mouse spleen, many dendritic cells and macrophages adhered to plastic, but in manually disrupted thymi, few adherent cells were evident as reported (29). Thymus suspensions did contain nonadherent Ia$^+$ small lymphocytes, as detected by immunofluorescence with FITC–anti-I-A. Most of these cells were Ig$^+$. When B cells were removed with anti-Ig and complement, Ia$^+$,Ig$^-$ cells remained that were larger and more irregular in shape than small lymphocytes. The Ia$^+$,Ig$^-$ cells accounted for 0.1–0.3% of thymus suspensions and were enriched in a low-density subpopulation that was depleted of most T cells with mAb to CD4 and CD8. The double-negative Ig$^-$ populations accounted for ~2% of thymus suspensions and contained 2–10% Ia$^+$ cells. 60–70% of the Ia$^+$ cells were killed by 33D1 anti–dendritic cell mAb and complement and >90% by anti-Ia; none were

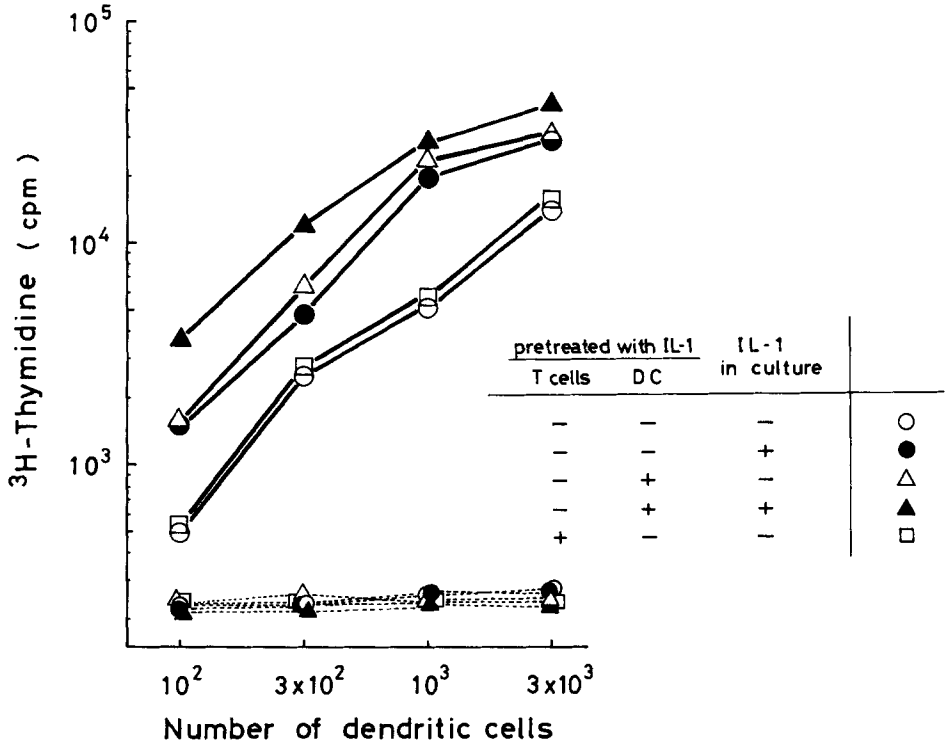

FIGURE 2. Pulsing dendritic cells with IL-1 amplifies their accessory function for thymocyte mitogenesis. Enriched populations of splenic dendritic cells, or nylon wool–nonadherent Ia$^-$ thymocytes, were pretreated for 16 h with or without 50 U/ml rIL-1α (see symbols). The dendritic cells and thymocytes were washed and cultured together with or without additional IL-1. 1 μg/ml Con A was present in some cultures (——) but not others (– – –). Note that Il-1 does not amplify the response when used to pretreat thymocytes, but does amplify dendritic cell function to the same extent as continuous IL-1.

killed by anti-Ig or anti-Thy-1 (Fig. 3, *top*). The Ia$^+$ cells had the same irregular shape and size as splenic dendritic cells (Fig. 3, *bottom*). The dendritic cell–enriched preparations lacked cells that could bind antibody-coated red cells or be stained with Mac-1 mAb (macrophages).

Irradiated, partially enriched thymic dendritic cells supported thymocyte mitogenesis to Con A, and to Con A plus IL-1 (Fig. 4), and also stimulated the MLR (not shown). The accessory function was 50-fold greater than unfractionated thymus (not shown). >90% of the accessory activity was removed by treatment with anti-Ia and complement (Fig. 4), and 60–70% with anti–dendritic cell mAb (not shown). We conclude that thymus contains very small numbers of dendritic cells. These are enriched in double-negative fractions and are active accessory cells for the mitogenesis of mature T cells and thymocytes.

IL-1 Induces the Production of Dendritic Cells from Ia$^-$ Precursors. Low-density, CD4$^-$, CD8$^-$, Ig$^-$ thymus cells were cultured 1–2 d with 50 U/ml rIL-1, washed, and used as accessory cells. As a control, double-negative populations were depleted of dendritic cells with anti-Ia and complement and cultured with IL-1. The latter, Ia$^-$, control cultures initially lacked dendritic cells (Fig. 3) but

TABLE II
Effect of Anti-IL-1 on Thymocyte Mitogenesis

Group	Pretreatment of spleen dendritic cells	Addition to thymocyte assay:		Mitogenesis ([^{3}H]TdR incorporation) to dendritic cell doses of:			
		rIL-1	anti-IL-1	100	300	1,000	3,000
				cpm × 10^{-3}			
1	None	−	−	0.6	1.8	6.1	14.2
2		−	+	0.6	1.7	5.7	13.3
3		+	−	1.3	4.7	13.8	39.4
4		+	+	0.5	2.2	6.2	17.4
5	50 U/ml IL-1	−	−	4.0	13.1	26.9	57.0
6		−	+	3.6	11.6	23.8	53.7
7		+	−	5.6	17.3	30.7	60.9
8		+	+	3.5	12.1	28.6	59.5

Thymus suspensions were depleted of endogenous accessory cells by passage over nylon wool and treatment with anti-Ia and complement. Splenic dendritic cells were added to the thymocytes in graded doses. The dendritic cells were cultured overnight with or without IL-1 and washed before being added to the thymocyte assay. The assays were supplemented with additional rIL-1 (50 U/ml) or anti-IL-1 (5 μg/ml) as indicated. All data are shown for responses in the presence of 1 μg/ml Con A. Note that pretreatment of dendritic cells with IL-1 enhanced function 10-fold (group 1 vs. 5), and that anti-IL-1 blocked the effect of exogenous IL-1 in the thymocyte assay (group 3 vs. 4) but did not block the function of dendritic cells that were pulsed with IL-1 (groups 5–8).

regenerated them in the presence of IL-1. The new Ia^+ population had the same phenotype as fresh thymic dendritic cells (data not shown, but identical to Fig. 3). Two-thirds of the Ia^+ cells were sensitive to lysis with anti–dendritic cell mAb but not with anti-Ig or anti-Thy-1 antibody and complement. The development of Ia^+ dendritic cells from Ia^- cultures was accompanied by the development of significant stimulating activity in the MLR (Fig. 5, *left*). The stimulators were primarily Ia^+; $33D1^+$, and Fc receptor–negative (Fig. 5, *right*).

In other experiments (not shown), we noted that the induction of Ia^+ dendritic cells by IL-1 required 2 d and occurred only in the thymic low-density, double-negative fraction. Dendritic cells did not develop from high-density fractions, or from bone marrow suspensions. We were unable to detect Ia^- dendritic cell precursors in spleen and lymph node. In these organs, double-negative, Ia^- fractions represented <1% of total cells and were primarily macrophages. Pretreatment with 900 rads of ^{137}Cs did *not* block the development of thymic dendritic cells from Ia^- precursors. Other recombinant cytokines (IL-2, IL-3, and GM-CSF) induced few and sometimes no dendritic cells from Ia^- cultures. We conclude that thymus has a trace group of Ia^- precursors that give rise to typical Ia^+ dendritic cells upon culture in IL-1.

IL-1 Enhances the Function of Thymic DC in Thymocyte Mitogenesis. Low-density, double negative, Ia^- or Ig^- thymus cultures were maintained for 1–3 d with or without human or mouse rIL-1. The populations were washed and added in graded doses to thymocyte mitogenesis assays. Pretreatment with IL-1 enhanced the accessory function of the double-negative, Ig^- cultures 10-fold (Table III, compare groups A and B). Populations that had been depleted of dendritic cells

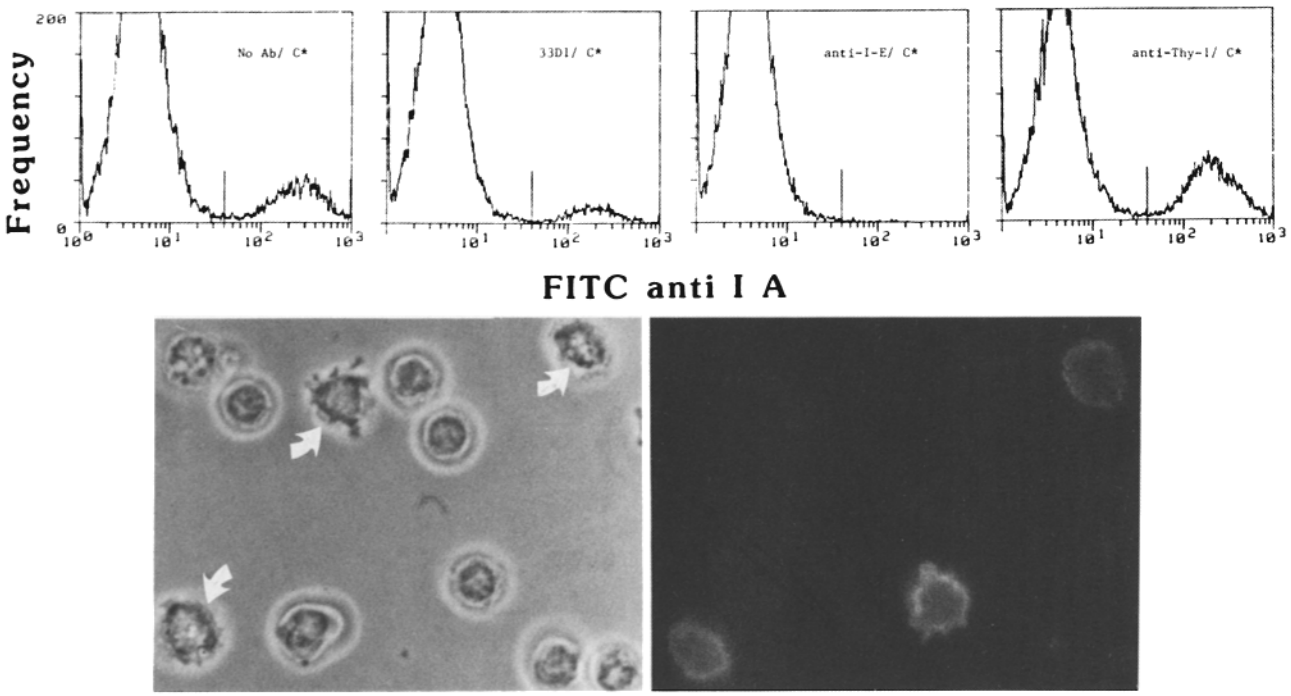

FIGURE 3. Ia^+ dendritic cells in thymus. The trace fraction of thymic dendritic cells was enriched by preparing low-density suspensions that were depleted of $CD4^+$,$CD8^+$,Ig^+ lymphocytes. Suspensions were then treated a second time with no mAb, 33D1 anti–dendritic cell (33), 14-4-5S anti-I-E (22), HO 13-4 anti-Thy-1 (34) and complement as indicated, or anti-Ig (not shown, but the results were identical to the no antibody control). The cells were stained with FITC-B21-2), anti-I-A, and ethidium bromide before flow cytometry. Dead cells were gated by ethidium bromide staining. 10,000 live cells were analyzed on a FACS 440. The frequencies of Ia^+ cells were 12.6, 5.5, 0.2, and 22.5% from left to right respectively. Similar results were obtained in two other tests. Immunofluorescence and phase contrast microscopy (*bottom*) illustrate the appearance of thymic, Ia^+, dendritic cells (*arrows*). No staining was observed with a FITC–anti–human leukocyte mAb as control (not shown).

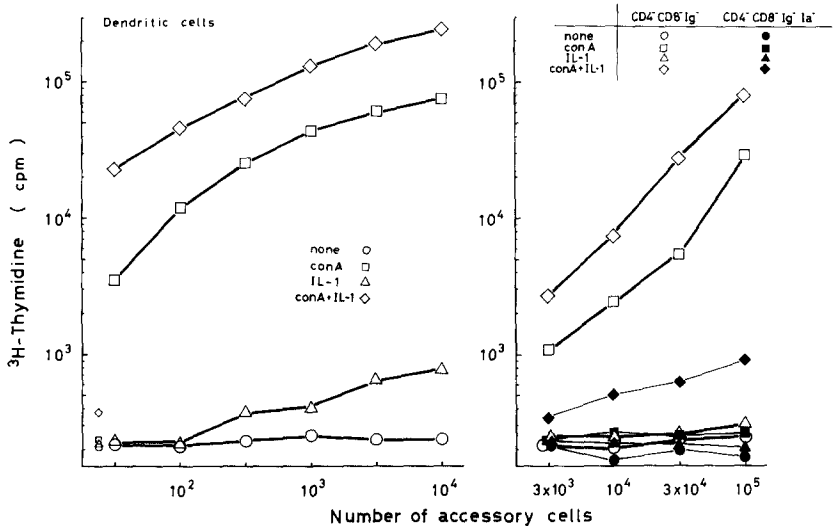

FIGURE 4. Function of dendritic cells in thymocyte mitogenesis. Enriched populations of splenic dendritic cells (*left*) were compared with partially enriched populations of thymic dendritic cells prepared as in Fig. 3 by depleting most T and B lymphocytes with anti-CD4,8 and Ig antibodies and complement (*right, open symbols*). Aliquots of the latter were also treated with anti-Ia and complement (*right, closed symbols*) to deplete dendritic cells (see Fig. 3). Each of the three populations were irradiated and then added to nylon wool–nonadherent, Ia^- thymocytes to test accessory function for mitogenesis to Con A with or without IL-1. Note that the function of spleen and thymus accessory cells (*open symbols*) is enhanced by exogenous IL-1, and that function is largely ablated by treatment with anti-Ia and complement (*right, closed symbols*).

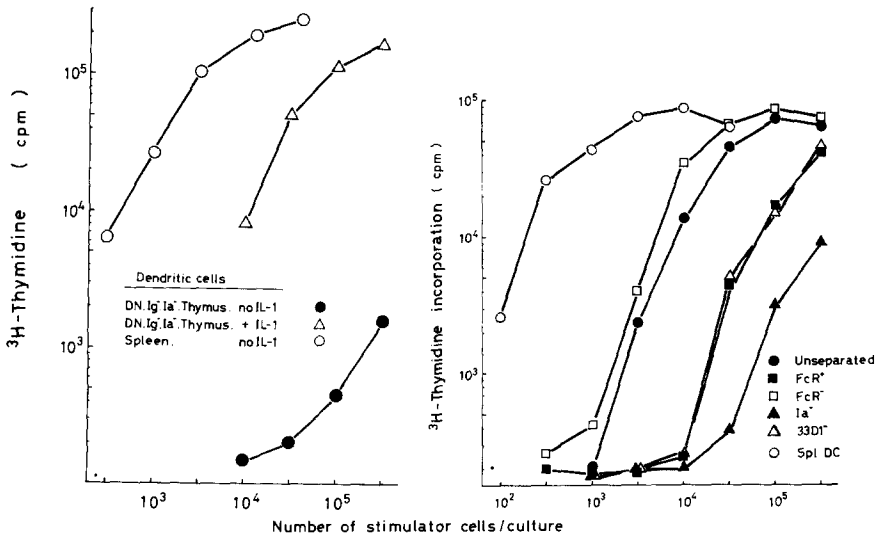

FIGURE 5. IL-1-induced development of MLR stimulating function from Ia^- precursors. (*Left*) Low-density, $CD4^-$,$CD8^-$ (double-negative or DN), Ig^-,Ia^- thymocytes were cultured 2 d with or without 50 U/ml IL-1. The cultures were washed and used to stimulate allogeneic, Ia^-, nylon-nonadherent spleen T cells. Note that IL-1 induces the development of MLR stimulating function from Ia^- precursors. (*Right*) Same as left, but the phenotype of the IL-1 induced cultures was studied. Aliquots were either separated into Fc receptor–positive and –negative fractions by rosetting with antibody-coated red cells (35), or treated with no mAb, 33D1 anti–dendritic cell (33), or anti-Ia antibody and complement as indicated.

TABLE III

Effect of Preculture with IL-1 on the Accessory Function of Thymic Dendritic Cells

Group	Source of accessory cells	Addition to thymocyte assay		Thymocyte mitogenesis with graded doses of accessory cells			
		IL-1	Con A	10^5	3×10^4	10^4	3×10^3
				cpm × 10^{-3}			
A1	Enriched thymic DC, cultured without IL-1	−	−	0.2	0.2	0.2	0.2
A2		+	−	0.4	0.3	0.2	0.2
A3		−	+	28.7	8.9	3.7	1.4
A4		+	+	82.4	26.3	14.1	6.0
B1	Enriched thymic DC, cultured with IL-1	−	−	0.8	0.2	0.2	0.2
B2		+	−	2.2	1.3	0.6	0.4
B3		−	+	157.5	55.8	25.0	8.7
B4		+	+	231.7	146.0	99.6	33.4
C1	Ia/C*-depleted thymic DC cultured without IL-1	−	−	0.3	0.3	0.2	0.2
C2		+	−	0.5	0.3	0.3	0.2
C3		−	+	0.3	0.3	0.3	0.2
C4		+	+	0.7	0.5	0.4	0.3
D1	Ia/C*-depleted thymic DC cultured with IL-1	−	−	0.2	0.2	0.2	0.2
D2		+	−	1.7	0.7	0.4	0.3
D3		−	+	106.1	23.3	8.4	2.6
D4		+	+	147.4	53.3	32.0	10.2

Thymic dendritic cells (DC) were partially enriched by treating low-density thymocytes with a combination of anti-CD4, CD8, and Ig antibodies and complement. The cells were cultured 3 d at 3 × 10^6 cells/16-mm well with or without 50 U/ml murine rIL-1α (groups A and B). Replicates were treated with anti-Ia and complement (in addition to the above antibodies; groups C and D). The four types of culture were washed, counted, and added in graded doses to 3 × 10^5 nylon nonadherent, Ia^- thymocytes. The mitogenesis assay was performed in the presence or absence of IL-1 and Con A as indicated. Note that IL-1 increases accessory function about 10-fold (group A vs. B), and induces the formation of accessory cells from Ia^- precursors (group C vs. D).

with anti-Ia and complement had no accessory function (group C). However, when these same cultures were supplemented with IL-1, significant accessory function developed (group D).

Antibody- and complement-mediated cytolysis was used to verify that the IL-1-induced accessory cells had the phenotype of dendritic cells. For both Con A, and Con A plus IL-1 responses, accessory cell function was reduced by >90% with anti-Ia and 60–70% with anti–dendritic cell mAb and complement, but not by anti-Ig (Fig. 6).

Since our initial experiments revealed significant accessory function in Ia^- thymocytes that was removed by passage over nylon wool (Table I), we tested if IL-1 would induce dendritic cells from nylon wool–nonadherent fractions. Few were detected either by immunofluorescence (not shown) or by measurements of accessory function (Fig. 7). Therefore the nylon wool–adherent fraction of thymus contains an Ia^- precursor that requires IL-1 to develop the typical features of lymphoid dendritic cells.

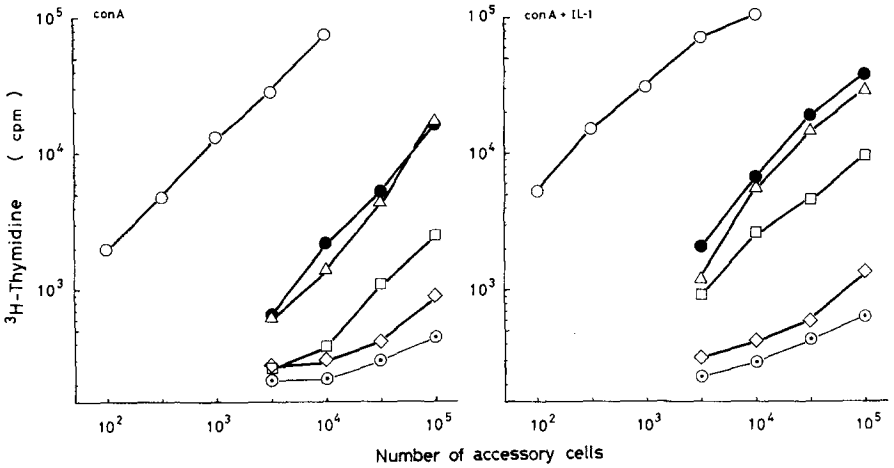

FIGURE 6. Phenotype of IL-1-induced accessory cells for thymocyte mitogenesis. Low-density, double-negative thymocytes were depleted of Ia$^+$ cells as in Figs. 5 and 6 and cultured 3 d in medium with (●) or without (⊙) 50 U/ml rIL-1α. The cells were treated with mAb and complement, washed, irradiated (900 rad), and tested for accessory function in the thymocyte mitogenesis response to Con A (*left*) or to Con A plus IL-1 (*right*). Untreated spleen dendritic cells were used as a positive control (○). The mAb were none (●), anti-μ (△), 33D1 anti-dendritic cell (□), and 14-4-5S anti-I-E (◇). Note that IL-1 induces accessory function (compare ● and ⊙), and that these accessory cells are sensitive to lysis with anti-Ia and anti-dendritic cell, but not anti-Ig and complement. The difference in the efficacy of killing with anti-Ia (B21-2) and anti-dendritic cell (33D1) mAb probably reflects the difference in the levels of the corresponding antigens. Quantitative binding (33) and flow cytometry (not shown) indicate that the number of B21-2 binding sites is 20-fold higher than 33D1.

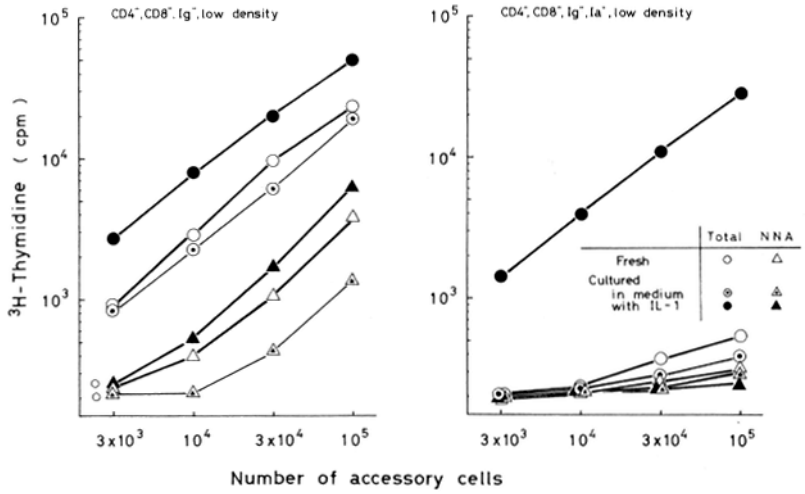

FIGURE 7. IL-1-dependent, Ia$^-$ dendritic cell precursors are depleted by passage over nylon wool. Low-density, CD4$^-$,CD8$^-$ thymocytes were prepared and depleted of B cells (Ig$^-$; *left*) or both B cells and dendritic cells (Ig$^-$,Ia$^-$; *right*). Each fraction was then studied with or without passage over nylon wool, and with or without culture for 3 d in medium or in medium plus IL-1 (see legend). If dendritic cells were present initially (*left*), it is evident that preculture in IL-1 enhanced accessory function (compare ⊙ and ●), and that many of the accessory cells are initially nylon wool–adherent (compare *circles* and *triangles*). If dendritic cells were depleted from the starting population (*right*), accessory function was partially restored by culture in IL-1 (●). The IL-1-responsive cells were nylon wool adherent (▲).

Discussion

These results on thymocyte proliferation to Con A, and to Con A plus IL-1, represent a beginning for studying the contributions of dendritic cells to thymus function in culture. There has been considerable emphasis on the importance of bone marrow–derived, non-T cells in thymus biology. However the function of endogenous dendritic cells in thymocyte responses has not been explored directly. The current study shows that two pools of dendritic cells exist in thymus, Ia^+ and Ia^-. Both are very active in supporting thymocyte proliferation to Con A, and IL-1 amplifies their function. Very few macrophages are detected in thymus suspensions. However, peritoneal macrophages are much less active than dendritic cells in responses to lectin, as reported (28). The function of macrophages cannot be enhanced by exogenous IL-1 (Fig. 1).

The difficulty in studying dendritic cells in teased thymic suspensions is that the frequency is low (0.1–0.3%), and the dendritic cells are nonadherent. We have been unable to enrich these dendritic cells to a high degree of purity (Fig. 3). Nonetheless, the data on partially enriched populations indicate that thymic and splenic dendritic cells are similar in phenotype and function. In recent experiments, we have dissociated thymi with collagenase to obtain *adherent* dendritic cells and macrophages that are very similar to those found in spleen (30). The monolayers can be used to prepare highly enriched fractions of thymic dendritic cells and macrophages. Adherence-derived dendritic cells behave similarly in many respects to the nonadherent dendritic cells described here. In this study we have focused on manually disrupted thymi, since this is the standard source of cells for most studies of thymocyte function.

Prior work has shown that IL-1 amplifies *peripheral* T cell mitogenesis by enhancing dendritic cell function (18). This also seems to be the case for the thymocyte response to lectin plus IL-1, which is the classical bioassay used to detect the cytokine (17). When IL-1 is used to pretreat dendritic cells, the populations can be washed and will exhibit functional activity that is 3–10-fold enhanced over untreated populations (Figs. 2 and 4; Table III, groups A,B). The enhanced function resists neutralizing anti-IL-1 antibodies (Table II), indicating that cytokine is not being carried over into the mitogenesis assay. Further work is needed to test if IL-1 can have additional direct effects on some thymocytes, but it appears that a major site of action of IL-1 in lectin responses is at the level of dendritic cells.

When IL-1 is used to pretreat *Ia^-* thymus fractions, the amplifying effect of cytokine is qualitatively and quantitatively more dramatic (Table III; Figs. 5–7). No accessory function is seen in the absence of cytokine pretreatment, while active dendritic cells develop in its presence. Bowers and Berkowicz (31) described Ia^- dendritic cell precursors in rat bone marrow maintained in serum free medium. To date we have not detected a similar population in mouse marrow.

There is a prior report which may indicate a similar role for IL-1 in intact thymus to that described here. DeLuca and Mizel (32) reported that anti-I-A mAb inhibited the recovery of functional T cells from fetal organ cultures. Exogenous rIL-1 reversed the block. Perhaps IL-1 induced the formation of needed accessory dendritic cells from Ia^- precursors.

Summary

The response of thymocytes to lectin is a standard tissue culture model for identifying cytokines such as IL-1 that are required for thymocyte mitogenesis. To study accessory cell requirements for these responses, it was necessary to deplete *endogenous* accessory cells with two techniques: anti-Ia and complement, and passage over nylon wool. Proliferation to Con A was then restored with 0.1–0.3% exogenous splenic dendritic cells, or 30-fold higher levels of peritoneal macrophages. The "costimulatory" action of IL-1, whereby responses to lectin were enhanced 3–10-fold, required the presence of dendritic cells. This effect of IL-1 could be reproduced by culturing the dendritic cells for 12 h in 1 U/ml human or murine rIL-1α *before* addition to the thymocyte proliferation assay. The function of IL-1-treated dendritic cells was not blocked by a neutralizing anti-IL-1 antibody.

The endogenous population of thymic accessory cells was partially characterized. A trace (0.1–0.3%) fraction of Ia^+,Ig^-, plastic nonadherent dendritic cells was visualized and enriched to a level of 1–10% by depleting $CD4^+$,$CD8^+$, and Ig^+ lymphocytes. When this double-negative population was cultured with IL-1 and washed, the treated *thymic* dendritic cells were 10-fold more active as accessory cells. When the $CD4^-$,$CD8^-$,Ig^- populations were depleted of dendritic cells with anti-Ia and complement, the subsequent addition of IL-1 had a second effect. Ia^+ dendritic cells redeveloped over a 2-d interval, and they exhibited the same properties as resident dendritic cells in thymus and spleen. The majority were lysed by 33D1 anti–dendritic cell mAb and complement, lacked Fc receptors, and acted as powerful stimulators of the MLR and Con A mitogenesis. The development of dendritic cells did not occur with IL-2, -3, -4 or granulocyte/macrophage colony-stimulating factor or in nylon-nonadherent populations. The IL-1-dependent, Ia^- precursor was not detectable in bone marrow.

These results begin to analyze the endogenous accessory function of the thymus in culture. Dendritic cells actively stimulate thymocyte mitogenesis. The mitogenic action of IL-1 involves effects on resident Ia^+ dendritic cells as well as a new population of thymic, Ia^- precursors.

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References

1. Matsuyama, M., M. N. Wiadrowski, and D. Metcalf. 1966. Autoradiographic analysis of lymphopoiesis and lymphocyte migration in mice bearing multiple thymus grafts. *J. Exp. Med.* 123:559.
2. Shortman, K., and H. Jackson. 1974. The differentiation of T lymphocytes. I. Proliferation kinetics and interrelationships of subpopulations of mouse thymus cells. *Cell. Immunol.* 12:230.
3. Bryant, B. J. 1972. Renewal and fate in the mammalian thymus: mechanisms and inferences of thymocytokinetics. *Eur. J. Immunol.* 2:38.

4. Borum, K. 1973. Cell kinetics in mouse thymus studied by simultaneous use of ^{3}H-thymidine and colchicine. *Cell Tissue Kinet.* 6:545.
5. Claesson, M. H., and N. R. Hartmann. 1976. Cytodynamics in the thymus of young adult mice: a quantitative study on the loss of thymic blast cells and non proliferative small thymocytes. *Cell Tissue Kinet.* 9:273.
6. Penit, C. 1986. In vivo thymocyte maturation. Budr labeling of cycling thymocytes and phenotypic analysis of their progeny support the single lineage model. *J. Immunol.* 137:2115.
7. Kappler, J. W., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. *Cell.* 49:273.
8. Lo, D., and J. Sprent. 1986. Identity of cells that imprint H-2 restricted T-cell specificity in the thymus. *Nature (Lond.).* 319:672.
9. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* 148:766.
10. Oliver, P. D., and N. M. LeDourain. 1984. Avian thymic accessory cells. *J. Immunol.* 132:1748.
11. Kyewski, B. A., C. G. Fathman, and R. V. Rouse. 1986. Intrathymic presentation of circulating non-MHC antigens by medullary dendritic cells. An antigen-dependent microenvironment for T cell differentiation. *J. Exp. Med.* 163:231.
12. Barclay, A. N., and G. Mayrhofer. 1981. Bone marrow origin of Ia-positive cells in the medulla of rat thymus. *J. Exp. Med.* 153:1666.
13. Wong, T. W., W. E. F. Klinkert, and W. E. Bowers. 1982. Immunological properties of thymus cell subpopulations: rat dendritic cells are potent accessory cells and stimulators in a mixed leukocyte culture. *Immunobiology.* 160:413.
14. Pelletier, M., C. Tautu, D. Landry, S. Montplaisir, C. Chartrand, and C. Perreault. 1986. Characterization of human thymic dendritic cells in culture. *Immunology.* 58:263.
15. Steinman, R. M., K. Inaba, G. Schuler, and M. D. Witmer. 1986. Stimulation of the immune response: contributions of dendritic cells. *In* Mechanisms of Host Resistance to Infectious Agents, Tumors, and Allografts. R. M. Steinman and R. J. North, editors. The Rockefeller University Press, New York. 71.
16. Steinman, R. M., K. Inaba, G. Schuler, M. D. Witmer, S. Koide, E. Flechner, N. Bhardwaj, and J. W. Young. 1986. The role and mechanism of action of dendritic cells in transplantation immunity. *In* Progress in Immunology. Vol. VI. B. Cinader and R. G. Miller, editors. Academic Press, New York. 1013.
17. Gery, I., and B. H. Waksman. 1972. Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediators. *J. Exp. Med.* 136:143.
18. Koide, S. L., K. Inaba, and R. M. Steinman. 1987. Interleukin-1 enhances T-dependent immune responses by amplifying the function of dendritic cells. *J. Exp. Med.* 165:515.
19. Lomedico, P. T., U. Gubler, C. P. Hellman, M. Dukovich, J. G. Giri, Y.-C. E. Pan, K. Collier, R. Semionow, A. O. Chua, and S. B. Mizel. 1984. Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli. Nature (Lond.).* 312:458.
20. Kupper, T. S., D. W. Ballard, A. O. Chua, J. S. McGuire, P. M. Flood, M. C. Horowitz, R. Langdon, L. Lightfoot, and U. Gubler. 1986. Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1α and β mRNA. Keratinocyte epidermal cell-derived thymocyte-activating factor is identical to interleukin 1. *J. Exp. Med.* 164:2095.
21. Steinman, R. M., N. Nogueira, M. D. Witmer, J. D. Tydings, and I. S. Mellman. 1980. Lymphokine enhances the expression and synthesis of Ia antigen on cultured mouse peritoneal macrophages. *J. Exp. Med.* 152:1248.

22. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigen. *J. Immunol.* 124:533.
23. Witmer-Pack, M. D., J. Valinsky, W. Olivier, and R. M. Steinman. 1987. Quantitation of surface antigens on cultured murine epidermal Langerhans cells: rapid and selective increase in the level of surface MHC products. *J. Invest. Dermatol.* In press.
24. Nussenzweig, M. C., and R. M. Steinman. 1980. Contributions of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196.
25. Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK-1.5: similarity of L3T4 to the human leu3/T4 molecule. *J. Immunol.* 131:2445.
26. Raulet, D. H., P. D. Gottlieb, and M. J. Bevan. 1980. Fractionation of lymphocyte subpopulations with monoclonal antibodies specific for Lyt-2.2 and Lyt-3.1. *J. Immunol.* 125:1136.
27. Nussenzweig, M. C., R. M. Steinman, J. C. Unkeless, M. D. Witmer, B. Gutchinov, and Z. A. Cohn. 1981. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J. Exp. Med.* 154:168.
28. Hirayama, Y., K. Inaba, S. Komatsubara, K. Yoshida, J. Kawai, K. Naito, and S. Muramatsu. 1987. Accessory cell functions of dendritic cells and macrophages in the thymic T cell response to concanavalin A. *Immunology.* In press.
29. Steinman, R. M., and Z. A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 137:1142.
30. Steinman, R. M., and Z. A. Cohn. 1974. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J. Exp. Med.* 139:380.
31. Bowers, W. E., and M. R. Berkowitz. 1986. Differentiation of dendritic cells in cultures of rat bone marrow cells. *J. Exp. Med.* 163:872.
32. DeLuca, D., and S. B. Mizel. 1986. I-A-positive nonlymphoid cells and T cell development in murine fetal thymus organ cultures: interleukin 1 circumvents the block in T cell differentiation induced by monoclonal anti-I-A antibodies. *J. Immunol.* 137:1435.
33. Nussenzweig, M. C., R. M. Steinman, M. D. Witmer, and B. Gutchinov. 1982. A monoclonal antibody specific for mouse dendritic cells. *Proc. Natl. Acad. Sci. USA.* 79:161.
34. Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter. 1979. Properties and applications by monoclonal antibodies directed against determinants of the thy-1 locus. *J. Immunol.* 122:2491.
35. Steinman, R. M., G. Kaplan, M. D. Witmer, and Z. A. Cohn. 1979. Identification of a novel cell-type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J. Exp. Med.* 149:1.