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Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice

(major histocompatibility complex/T and B lymphocytes/macrophages/adherent cells)

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ABSTRACT Dendritic cells (DCs) are a new cell type initially identified in mouse lymphoid organs. Recently, DCs have been purified from mouse spleen. This paper demonstrates a functional role of DCs: they are potent stimulators of the primary mixed leukocyte reaction (MLR). As few as 300–1000 DCs doubled the proliferative activity of 5×10^6 allogeneic responder spleen cells, while $0.3\text{--}1.0 \times 10^5$ DCs induced a maximal stimulation of 30- to 80-fold. Between these extremes, the log of the MLR response increased linearly with the log of DC numbers. This dose-response assay was then used to compare the potency of purified DCs with that of other heterogeneous lymphoid populations, many of which gave dose-response curves with similar slopes. The potency of purified DCs as MLR stimulators was 100–300 times greater than that of unfractionated spleen cells. When spleen cells were fractionated by simple physical techniques, MLR-stimulating capacity in the subpopulations correlated closely with DC numbers. Removal of splenic B or T lymphocytes, by anti-immunoglobulin or anti-brain serum plus complement, did not reduce MLR-stimulating capacity. Finally, several populations, enriched in mononuclear phagocytes but lacking in DCs, stimulated weakly if at all. We conclude that DCs are a potent stimulating cell and are at least 100 times more effective than other major cell subclasses—i.e., B and T lymphocytes and macrophages.

Lymphoid dendritic cells (DCs) are a novel population of cells identified initially on the basis of distinctive cytologic criteria (1–4). DCs occur in low frequency, accounting for 1% or less of nucleated cells in peripheral lymphoid organs. They are readily identified in mouse spleen suspensions, where 80% or more adhere and spread on glass surfaces (1). DCs are derived from bone marrow precursors and increase in numbers during the first few weeks of life (3). They lack most of the differentiation markers of other lymphoid cell types, especially mononuclear phagocytes and lymphocytes (2). However, recently we have documented that DCs express both H-2 and Ia alloantigens to the same extent as other reactive spleen cells.* We have also been able to purify splenic DCs, to maintain them *in vitro* for several days, and to show that they remain distinctive in appearance and surface markers.* In this paper, we demonstrate that small numbers of purified DCs reliably induce a vigorous proliferative response in allogeneic T cells, the hallmark of a primary mixed leukocyte reaction (1° MLR). We then show that the efficacy of various lymphoid organs and subpopulations in stimulating an MLR appears to depend in large part on their content of DCs.

MATERIALS AND METHODS

Mice. DBA/2, C57BL/6, CD2F₁ (BALB/c \times DBA/2), and B6D2F₁ (C57BL/6 \times DBA/2) mice were obtained from the Trudeau Institute, Saranac Lake, NY; Flow Laboratories, Dublin, VA; and The Jackson Laboratory, Bar Harbor, ME. Mice 6 weeks to 6 months in age, of both sexes, were used.

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Antisera. Rabbit anti-mouse brain serum, an anti-T cell reagent, was obtained from Accurate Scientific, Hicksville, NY. This serum: killed 20–30% of spleen cells in the presence of guinea pig complement (Cordis Laboratories, Miami, FL); totally abolished the mitogen response to concanavalin A without altering lipopolysaccharide responses; and killed 85% of cells proliferating in MLRs, without killing lipopolysaccharide-induced lymphoblasts, both obtained as previously described (5). Rabbit anti-mouse κ serum was prepared as described (6). This serum: killed 50–60% of total spleen cells; removed more than 80% of surface immunoglobulin-bearing cells (stained by indirect immunofluorescence); abolished more than 80% of the total lipopolysaccharide response; and selectively immunoprecipitated surface immunoglobulin from radioiodinated lymphocytes (W. van Voorhis, personal communication). An anti H-2b serum was obtained from the Research Resources Branch, National Institutes of Health. This serum (labeled D-2) was directed to private specificity 2, of the D end of the H-2b haplotype. In the presence of complement, D-2 killed 100% of H-2b cells and no H-2d cells.

Lymphoid Cells. Spleen, mesenteric lymph node, and thymus were teased apart and further disrupted on a stainless steel sieve. Resident peritoneal cells were aspirated from the abdominal cavity, bone marrow cells were flushed from femurs, and peripheral blood leukocytes were isolated on Ficoll/Hypaque (Pharmacia, Piscataway, NJ). Cells yields were $6\text{--}15 \times 10^7$ per spleen, $1\text{--}3 \times 10^7$ per node, $10\text{--}15 \times 10^7$ per thymus, 0.5×10^7 per femur, $3\text{--}6 \times 10^6$ per peritoneal cavity, and 5×10^6 per ml of blood.

Adherent Cells. Samples (0.05–0.15 ml) of lymphoid cells at $0.2\text{--}2.0 \times 10^7$ /ml were applied in 1% mouse serum/RPMI 1640 culture medium (Grand Island Biologicals, Grand Island, NY) to 15-mm circular glass coverslips for 1–2 hr at 37°. Nonadherent cells were dislodged with pasteur pipettes. The coverslips with adherent cells were then transferred to the wells in which MLRs were induced. To enumerate and identify glass-adherent cells, $2\text{--}5 \times 10^5$ cells were spun into 13-mm circular coverslips as described (5), and allowed to adhere as above. Cyto centrifugation produced a uniform distribution of adherent cells that could be fixed in 1.25% buffered glutaraldehyde and examined by phase contrast microscopy.

The adherent spleen population we studied consisted primarily of DCs and macrophages, which could be identified by cytologic and other criteria (1, 2, *). DCs flatten and extend cell processes in several directions when adherent to glass. The nucleus is large and irregular in shape, has a refractile appearance due to a peripheral rim of heterochromatin, and has small nucleoli. DCs contain many spherical, phase-dense

Abbreviations: MLR, mixed leukocyte reaction; MHC, major histocompatibility complex; DC, dendritic cells; sRBC, sheep erythrocytes.

* Steinman, R. M., Kaplan, G., Witmer, M. D. & Cohn, Z. A., *J. Exp. Med.*, in press.

granules that are mitochondria, but these cells lack the many pinocytic vesicles, lysosomes, and surface ruffles characteristic of macrophages. Splenic macrophages in our preparations are usually small, ruffled cells that are best identified by the ability to interiorize sheep erythrocytes opsonized with hyperimmune antiserum to sheep erythrocytes. These cells develop into much larger and more actively phagocytic macrophages after 2–3 days of tissue culture. Adherent peritoneal cells consist almost entirely of macrophages, and DCs are extremely rare (less than 0.1%). An exception occurred with a shipment of B6D2F₁ mice in which 1–5% of the adherent peritoneal cells were DCs (see *Results*).

Some DCs in spleen and peritoneal cavity do not adhere to glass, but will attach and spread in a typical fashion on coverslips coated with poly(L-lysine) (Sigma Chemical, St. Louis, MO; 25 μ g/ml of phosphate-buffered saline for 20 min at room temperature).

Purification of Dendritic Cells. The criteria for monitoring the purification of DCs are described in detail elsewhere.* The purification protocol itself involves four steps. A low-density subpopulation of spleen was obtained by floatation on dense bovine plasma albumin (Armour Pharmaceuticals, Phoenix, AZ) columns, density = 1.080 g/cm³, and then a glass-adherent, low-density fraction was prepared (2). Most DCs floated on the bovine plasma albumin columns, and some 80% adhered to glass. The main, if not only, contaminating cells at this stage were immature macrophages. The third step was to culture the cells overnight in RPMI 1640 medium supplemented with penicillin and 50 μ M 2-mercaptoethanol and serum—either 2.5% heat-inactivated mouse serum or 5% fetal calf serum. During this period, most of the original adherent population eluted from, or could easily be dislodged from, the glass surface, yielding a 90% trypan-blue-negative cell suspension. To remove macrophages from the eluted cells, rosettes were formed with heavily opsonized sheep erythrocytes (sRBC). A 5% (vol/vol) suspension of sRBC was opsonized with an equal volume of a 1:60 dilution of hyperimmune, rabbit anti-sRBC; a 1:40 dilution under these conditions produced hemagglutination. The opsonized sRBC were mixed with white cells at a ratio of 30:1, spun into a pellet, and allowed to rosette at 4°C, and then the rosettes were removed by centrifugation on dense bovine plasma albumin, 1.088 g/cm³. The floating population consisted of at least 95% DCs, while the pelleting cells contained the rosetted macrophages and 10–20% nonrosetted DCs. To identify DCs by cytologic criteria, it was necessary to spin the cells onto poly(L-lysine)-coated coverslips. Following attachment and glutaraldehyde fixation, the DCs exhibited irregular shapes, refractile and often contorted nuclei lacking large nucleoli, and phase-dense spherical granules that were mitochondria. Pinocytic vesicles, abundant lysosomes, and surface ruffling were absent. As described elsewhere,* the purified DCs were totally insensitive to treatment with anti-Ig and anti-T cell sera plus complement, did not rosette sRBC opsonized with antibody and complement and were all killed by specific anti-Ia or anti-H-2 sera plus complement.

Mixed Leukocyte Reaction (MLR). Cultures were set up in 16-mm diameter, flat-bottomed tissue culture wells (Rochester Scientific, Rochester, NY) in 1 mL of culture medium, with 1% heat-inactivated mouse serum and 50 μ M 2-mercaptoethanol as supplements. All cultures contained 4–5 $\times 10^6$ responder cells and variable numbers of stimulator cells (see *Results*). All stimulators were treated with mitomycin C (Sigma Chemicals), 25 μ g/ml, for 30 min at 37°C. Stimulation was performed across both allogeneic and semiallogeneic (F₁) barriers, using the mouse strains listed above.

Prior to assay, duplicate cultures were harvested with a pasteur pipette and spun at 200 $\times g$ for 10 min, and the cell

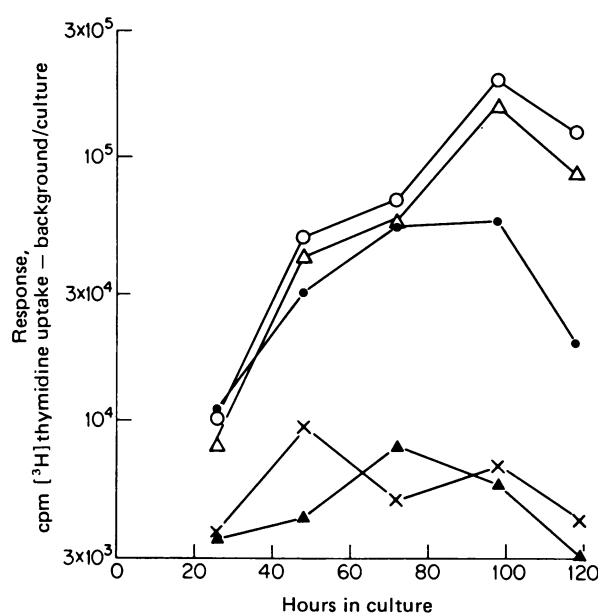


FIG. 1. Kinetics of the proliferative response ($[^3\text{H}]$ thymidine uptake/culture following a 2-hr pulse of 1.8 $\mu\text{Ci/ml}$) of 5×10^6 DBA/2 spleen cells with various sources of mitomycin-treated C57BL/6 stimulator cells: ○, 10^5 purified DCs; Δ, adherent cells from 3×10^6 low-density spleen cells containing about 10^5 DCs and "contaminated" with 10^5 immature macrophages; ●, 3×10^6 unfractionated spleen cells; ×, 7×10^5 adherent resident peritoneal cells, which are more than 90% macrophages and have rare DCs; ▲, no stimulator cells.

pellet was resuspended in 2 ml of fresh culture medium; 0.2 ml of the cell suspension was added in triplicate to Microtest wells (Rochester Scientific) followed by 0.05 ml of $[^3\text{H}]$ thymidine (Schwarz/Mann, Orangeburg, NY; specific activity, 6.0 Ci/mmol, final concentration 1.8 $\mu\text{Ci/ml}$). The cells were cultured an additional 2 hr and then harvested on a multisample harvester. Incorporated $[^3\text{H}]$ thymidine was measured with Aquasol (New England Nuclear, Boston, MA) as scintillant and a liquid scintillation counter operating at 42% efficiency. All data are given as means of the microtest assays. Standard deviations were generally less than 10% and always less than 20% of the mean.

RESULTS

DCs Stimulate an MLR. The first evidence that DCs could stimulate an MLR came from studies of adherent low-density spleen cells. Coverslips containing a mixture of 10^5 DCs and 10^5 macrophages induced a large and prolonged proliferative response in allogeneic spleen cells (Fig. 1). The response peaked at day 4, and stimulation ratios of 30- to 75-fold over the proliferative activity in unstimulated cells were obtained. The stimulation ratios were always greater than the ratio induced by 3×10^6 unfractionated spleen cells (Fig. 1), a typical optimal dose for the mouse MLR.

To determine if adherent macrophages or DCs were stimulating the MLR, we first examined macrophage-rich populations from other lymphoid organs. Adherent peripheral blood and bone marrow leukocytes, which contain mononuclear phagocytes in varying stages of development (7), were first tested but did not stimulate (data not shown). Adherent peritoneal cells, which generally consist of more than 90% macrophages and rare DCs, gave little or no stimulation of thymidine uptake (Fig. 1, Table 1). Significant stimulation was noted in one shipment of B6D2F₁ mice. However, these mice were unusual in that adherent peritoneal preparations contained DCs in numbers comparable to the number present in adherent

Table 1. MLR-stimulating capacity of subpopulations of peritoneal and spleen cells

Cells ($\times 10^{-6}$) to prepare stimulating subpopulation*	Response ($[^3\text{H}]$ thymidine uptake – background, cpm/culture) after stimulation with						
	Peritoneal cells, DBA/2		Adherent peritoneal cells, B6D2F ₁			Peritoneal cells, DBA/2	Low-density spleen cells, B6D2F ₁
	Adherent (1)	Nonadherent (2)	Group 1 (3)	Group 2 (4)	Group 3 (5)	Adherent (6)	Adherent (7)
1.0	8210	76,225	19,625	125,640	37,125	7050	116,150
0.3	7600	49,915	7,080	69,250	11,045	7125	64,500
0.1	1265	20,075	5,520	24,750	2,265	4490	17,850
DCs ($\times 10^{-4}$)†	<0.1	0.30	<0.1	2.8	0.15	<0.1	3.1
Macrophages ($\times 10^{-4}$)‡	35	5	30	35	35	40	3

Several experiments in which 5×10^6 C57BL/6 responder spleen cells were stimulated with mitomycin-treated DBA/2 or B6D2F₁ (DBA/2 \times C57BL/6) cells. The stimulations in columns 1 and 2 were simultaneous, as were those in columns 4, 6, and 7. In all cases, the background (unstimulated C57BL/6 cells) ranged from 4800 to 5100 cpm per culture.

* Dose used to prepare the stimulating cell population. About 35–40% of peritoneal cells adhere to glass vs. 8% for low-density spleen cells.

† Number of DCs enumerated by phase-contrast microscopy in the stimulating cell inoculum at the 1×10^6 dose.

‡ Number of macrophages enumerated by phase-contrast microscopy and phagocytosis of opsonized sRBC in the stimulating cell inoculum at the 1×10^6 dose.

low-density spleen cells (Table 1, columns 4 and 7). Taken together, the data in Table 1 indicate that MLR stimulation could not be correlated with the numbers of macrophages in the stimulating preparation.

We then purified DCs from adherent low-density spleen cells. DCs (10^5) stimulated an MLR identically to an equivalent number of adherent low-density DCs (Fig. 1). Similar stimulations were obtained with 10^5 purified DCs that had been maintained in culture for a total of 4 days. Also, mitomycin-treated DCs induced little (less than 2-fold boost in thymidine uptake) or no change in the proliferative activity of syngeneic spleen cells.

Proliferating Cells in the DC-Induced MLR. Cells labeled with $[^3\text{H}]$ thymidine at day 4 of a 1° MLR induced by 10^5 purified DCs had the cytologic features of lymphoblasts in autoradiograms of cell smears. These pulse-labeled cells—i.e., cells in S phase of the proliferative cycle—accounted for 20–50% of the $2.5\text{--}4.0 \times 10^6$ viable recovered cells. If the cultures were treated with antisera plus guinea pig complement prior to measurement of $[^3\text{H}]$ thymidine uptake, more than 85 and 95% of the proliferating cells, respectively, could be shown to carry the brain antigen(s) characteristic of T cells, and the H-2 antigens of the responder strain. We conclude that small numbers of purified lymphoid DCs induce a typical 1° MLR in alloreactive mouse spleen cells.

Potency of DCs in Inducing an MLR. Detailed dose-response curves were necessary to compare the potency of various cell populations to stimulate an MLR. We first looked at various doses of purified splenic DCs vs. whole or unfractionated spleen, using constant numbers of responder spleen cells (Fig. 2). As few as $0.3\text{--}1 \times 10^3$ purified DCs induced detectable proliferative activity—i.e., a stimulation ratio of 2—after 4 days of culture with 5×10^6 responder cells. The corresponding threshold dose for whole spleen was $0.3\text{--}3 \times 10^5$. With increasing doses of stimulators, the log of the proliferative response increased linearly with the log of the stimulating cell dose. The slopes for both purified DCs and unfractionated spleen were similar in all experiments assayed at day 4, indicating that the stimulating unit in both purified DCs and unfractionated spleen worked with similar efficacy (Fig. 2). However, the number of such stimulatory units, or potency, was very different. That is, if one determined the number of cells required for a given level of $[^3\text{H}]$ thymidine uptake on the linear portion of the dose-response curve, 1/100th to 1/300th as many purified DCs were needed in comparison to whole spleen. This observation has been made in nine different experiments using

either purified DCs or adherent low-density spleen cells. The maximum response induced by DCs was much greater than that induced by whole spleen, usually 2- to 4 fold. This response was obtained with $0.3\text{--}1 \times 10^5$ DCs vs. $1\text{--}3 \times 10^6$ unfractionated spleen cells. The ability of unfractionated stimulator spleen cells to induce an MLR was not enhanced by the addition of adherent responder low-density spleen cells containing 1×10^5 DCs (data not shown).

A remarkable feature of these dose-response data is that DCs comprise about 1/200th of normal nucleated spleen cells. Therefore the bulk of the stimulatory capacity in spleen could be ascribed to the presence of small numbers of DCs. Or, alternatively, non-DCs in spleen must be extremely weak MLR stimulators. The remainder of this paper looks at the efficacy of other lymphoid subpopulations and lymphoid organs to induce an MLR, using the dose-response assay described above (Fig. 2).

Ability of Additional Mouse Lymphoid Subpopulations to Induce an MLR. We first manipulated the frequency of DCs in various spleen cell subpopulations by simple physical techniques. Spleen cells were separated by floatation on dense bovine plasma albumin columns. The low-density fraction, which contained 15% of the total cells and most of the DCs, was 6 times more potent than unfractionated spleen in inducing an MLR,

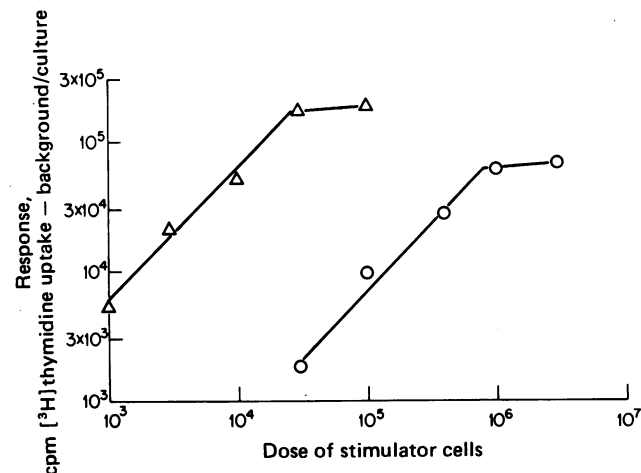


FIG. 2. Potency of purified DCs (Δ) vs. unfractionated spleen (\circ) in inducing an MLR. Responses (cpm of $[^3\text{H}]$ thymidine uptake – background/culture) were measured at various doses of stimulator cells, after 4 days of culture. Background was 5410 cpm.

Table 2. MLR-stimulating capacity of spleen subpopulations

Cell fraction	Total cells $\times 10^{-7}$	% of spleen cells	DCs/ nucleated cells, %	Potency, cells $\times 10^{-4}$
Unfractionated spleen	40	100	0.5	20
Low-density	6	15	3.0	3
High-density	33	83	0.1	72
Adherent low-density	0.6	1.5	44	4
Nonadherent low-density	4	10	1.1	11

The composition of the various spleen cell fractions used in the experiment in Fig. 3 is given. DCs were enumerated by cytologic criteria under phase-contrast microscopy. The potency of the various cell fractions is given as the number of cells needed to give a 2-fold boost in thymidine uptake. For adherent and nonadherent low-density cells, this value is the number of low-density cells used to prepare these fractions. For example, the actual number of adherent cells that induced a 2-fold boost in thymidine uptake was 4×10^3 vs. 4×10^4 .

whereas the high-density fraction was substantially depleted (Table 2 and Fig. 3). Low- and high-density cells contained similar numbers of B and T cells as measured by cytotoxicity assays (2). The glass-adherent portion of the low-density fraction, which contained 10% of the low-density cells and 80% of the DCs, exhibited 3 times the stimulating capacity of the nonadherent cells (Table 2 and Fig. 3). Neither adherent nor nonadherent low-density cells initially contain many mature macrophages (2). However, after 2–3 days of culture, large numbers of typical macrophages arise from the nonadherent fraction.*

To further examine the contribution of T or B lymphocytes as MLR stimulators, we removed these cells by treatment with anti-brain or anti-Ig serum and complement; these antisera kill most T and B cells, respectively. The nonlysed cells were used as stimulators, but at proportionately lower numbers than controls treated with complement alone, or neither antibody nor complement. The dose-response curves for all populations

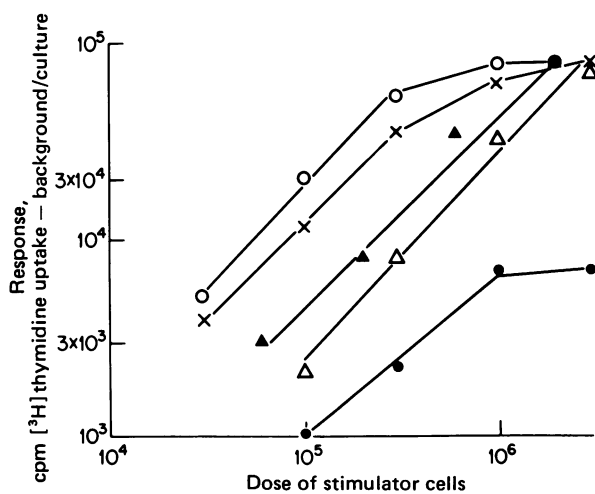


FIG. 3. Several subpopulations of B6D₂F₁ spleen cells were obtained and used at different doses to stimulate 5×10^6 C57BL/6 responder spleen cells for 4 days *in vitro*. Spleen cells (Δ) were first separated into low- (O) and high- (●) density fractions on dense bovine plasma albumin columns. Then various numbers of low-density cells were separated into glass-adherent (X) and nonadherent (\blacktriangle) fractions. The cell dose that is plotted is the dose used to prepare the adherent and nonadherent cells, and not the absolute number of stimulator cells used. The yields of total cells and DCs in these fractions are presented in Table 2.

Table 3. MLR-stimulating capacity after killing of B or T cells

Stimulating cell dose $\times 10^{-6}$	Response (cpm [3 H]thymidine uptake – background/culture) using spleen cells treated with			
	Culture medium	C only	Rabbit anti-mouse brain + C	Rabbit anti-mouse Ig + C
3	121,250	105,900	130,430	126,525
1	101,900	85,440	115,360	78,340
0.3	60,600	59,240	66,650	63,360
0.1	24,220	20,200	29,200	25,650

Mitomycin-treated spleen cells were exposed to culture medium only, guinea pig complement (C), anti-mouse brain serum plus complement (an anti-T cell reagent), or anti-mouse Ig plus complement. The stimulating cell dose is the number of cells present in the “no treatment” population. The actual numbers of cells in the C, anti-brain + C, and anti-Ig + C populations were 100%, 70%, and 50% of the number present in the no treatment group. MLRs were run for 4 days *in vitro* and background [3 H]thymidine uptake was 4675 cpm. This experiment has been performed four times with similar findings.

were strikingly similar (Table 3), indicating that many B or T cells can be removed without significantly decreasing the MLR stimulatory capacity of spleen.

Finally, we examined other lymphoid populations as stimulators (Table 4). Spleen and node both stimulated MLRs, with spleen being more effective than node. Both of these organs exhibit readily identifiable DCs (1). Thymus and bone marrow, which lack detectable DCs, stimulated poorly if at all. Purified allogeneic DCs (10^5) mixed with $1-3 \times 10^6$ allogeneic spleen, node, thymus, or bone marrow cells usually stimulated identically to purified DCs not mixed with other stimulators (data not shown), so we conclude that the latter populations do not contain inhibitors of the DC-induced MLR.

We conclude that the main ability of mouse lymphoid organs to stimulate an MLR does not reside in the major cell subclasses—i.e., B and T lymphocytes, and macrophages—but is due to the presence of small numbers of potent DCs.

DISCUSSION

A considerable literature exists on the ability of B and T lymphocytes and macrophages to stimulate the 1° MLR in mouse and in man (e.g., refs. 8–10). The data in this paper indicate that DCs may be 100 or more times more potent as stimulating cells than these other primary lymphoid subpopulations. First we noted that the MLR-stimulating capacity of spleen cell fractions, separated on the basis of simple physical techniques, correlated closely with content of DCs and not with numbers

Table 4. MLR-stimulating capacity of different lymphoid organs

Stimulating cells dose $\times 10^{-6}$	Response (cpm [3 H]thymidine uptake – background/culture) after stimulation with cells from			
	Spleen	Node	Thymus	Bone marrow
3	65,771	46,125	3655	0
1	51,300	11,906	785	4770
0.3	14,450	2,230	1050	3065
0.1	2,860	0	650	470

Various doses of mitomycin-treated B6D₂F₁ stimulating cells from four different lymphoid organs were added to a constant number (5×10^6) C57BL/6 responder spleen cells. Proliferative activity was measured 4 days later, after a 2-hr pulse of [3 H]thymidine at 1.8 μ Ci/ml. Background uptake in nonstimulated cultures was 4550 cpm.

of B and T lymphocytes or macrophages (Fig. 3 and Table 2). Killing most T or B cells in spleen altered stimulating capacity little if at all (Table 3). And data from lymphoid organs other than spleen—e.g., peritoneal cavity, marrow, thymus—suggested again that small numbers of DCs must be present for MLR stimulation to occur, and/or that DCs are far more powerful stimulators than these other cells (Tables 1 and 4). An assumption in our work is that the dose-response data obtained on purified DCs can be extrapolated to the behavior of DCs in heterogeneous stimulating mixtures. In most instances, we have not examined the stimulating capacity of purified lymphoid subpopulations directly, but data with such populations must take into account the possibility that trace "contamination" with DCs could occur.

The contribution made by small numbers of DCs to the MLR induced by heterogeneous cell mixtures must be studied further. The response itself must be characterized in cultures stimulated with purified DCs vs. whole spleen. Comparative information on the Ly phenotype of the responding cells, and the immunologic specificity of the 1° and 2° MLR will be important to acquire. We do know that the potency of purified DCs in inducing cytotoxic lymphocytes is 100 or more times greater than that of unfractionated spleen stimulators (M. Nussenzweig, personal communication). Another area in which more information is required is the criteria used to identify and enumerate DCs. These cells always occur in low frequency; they are primarily identified by a composite of morphologic features seen by phase contrast microscopy after spreading on glass; and we have no way of selectively and totally depleting DCs from a given population. These difficulties might be remedied by the availability of a specific anti-DC serum.

The mechanism whereby DCs act as such potent 1° MLR stimulators can only be considered in general terms. The amount, kind, and distribution of stimulating antigens may differ on DCs relative to other cell types. Current evidence is that the I subregion of the mouse major histocompatibility complex (MHC) encodes the antigenic determinants responsible for stimulating most of the proliferative activity in an MLR and that these determinants may correspond to the Ia alloantigens (11–14). We know that the cytotoxic and indirect immunofluorescence titers of anti-Ia, as well as anti-H-2, sera are similar towards DCs and other reactive spleen cells. Because the latter are relatively weak stimulators, we suspect that the expression of MHC-linked antigens is not solely responsible for MLR stimulation. Other possibilities exist. The responding lymphocyte may have to recognize something that exists in or on DCs in addition to the MHC antigens. Or DCs may influence the behavior of lymphocytes after the initial recognition event. After all, the MLR proliferative response we measure after 3–4 days of culture is a complex one in which cells must blast transform and continue to proliferate rather than die or enter a nondividing state.

The potency of DCs in inducing a 1° MLR has two additional implications. The first is whether DCs are present in other organs responsible for stimulating an MLR or even transplantation reaction *in situ*. It is possible that DCs and/or their equivalent are present in peripheral blood and in nonlymphoid organs, either as intrinsic cells or as "passenger leukocytes" (15). Because DCs are bone marrow derived (3), there must be a circulating phase whereby they gain access to lymphoid organs, and possibly to other organs and inflammatory sites. The lymphoid organs, such as spleen, that we study may be unusual only in the relative ease with which functioning DCs can be released and identified.

A second ramification of this paper is that DCs must be considered in experiments trying to unravel the normal or

physiologic function of the MHC. Considerable evidence has accumulated that the MHC functions in cell-cell interactions during many physiologic immune responses (e.g., refs. 16–18). In some systems, accessory non-T non-B cells seem to express this function of the MHC (16, 19–21), and most immunologists feel that the macrophage is the critical accessory cell. The evidence is neither direct nor clear. For example, relatively large numbers of macrophage-rich populations are required to achieve optimal effects (16), or peritoneal cells rich in macrophages do not function effectively (19, 20), or, in some accessory cell experiments, histocompatible macrophages are not required (22). DCs have been distinguished from macrophages in many significant parameters (23, *), including, in this paper, the ability to stimulate an MLR. It is possible that DCs have been present in accessory cell populations mediating MHC-linked effects. A critical point of this paper is that such small numbers of DCs are required for responses in alloreactive lymphocytes. We suggest that DCs and not macrophages will prove to be a critical accessory cell required in the generation of many immune responses.

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