INTERLEUKIN 1 PRODUCTION DURING ACCESSORY CELL-DEPENDENT MITOGENESIS OF T LYMPHOCYTES

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The concept that IL-1 is produced and is an essential cofactor for T cell proliferative responses to antigens has not been extensively explored with fresh populations of mononuclear cells. Recent studies in the mouse, with an IL-1α cDNA probe and rIL-1 have indicated that IL-1 is produced during monocyte but not dendritic cell-mediated, T cell proliferation (1). Furthermore, the activating properties of exogenous rIL-1 may be exerted at the level of the dendritic cell rather than the T cell (2).

We have studied IL-1 production during the human APC-T cell interaction. The human system is advantageous since one has access to monocytes and dendritic cells in blood, as well as a useful group of anti-IL-1 antibodies and antigen-specific clones. We find that IL-1 can be produced, but may not be essential for secondary T cell responses to antigen-bearing monocytes, and that there are MHC-restricted and nonrestricted pathways whereby T cells can induce IL-1 production by monocytes.

Materials and Methods

Culture Medium. Complete medium was RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5×10^{-5} M 2-ME, 20 μg/ml gentamycin (Gibco Laboratories), and 5–10% FCS (HyClone Laboratories, Logan, UT) or human AB^+ serum. FCS was used for OKT3 (anti-CD3)-induced T cell proliferative responses while AB^+ serum was used in all other circumstances.

Enrichment of Blood Mononuclear Cells, Monocytes, Dendritic Cells, and T Cells. Whole blood or human leukocyte fractions (New York Blood Center, New York, NY) were layered onto Ficoll Hypaque (Histopaque; Sigma Chemical Co., St. Louis, MO) and sedimented at 1,000 *g* for 20 min at 21°C. The mononuclear cells were washed twice in RPMI and studied immediately, or after 1–2 d in culture. Monocytes were enriched by adherence for 90 min at 37°C to plastic. The adherent cells were 55–85% monocytes by cytology and immunolabeling with the anti-CD14 mAb 3C10 (3). This antibody stains a majority (>95%) of blood monocytes and does not stain T cells, B cells, or dendritic cells (3). When highly enriched dendritic cells were required these were separated by a Percoll sedimentation method described elsewhere (4). The dendritic cells were obtained from the high density fraction by a multistep procedure involving depletion of T cells by sheep erythrocyte (E) rosetting, B cells by a density gradient, and residual monocytes by panning on dishes coated with human gamma globulin. To isolate enriched populations of B cells and dendritic cells together from PBMC, T cells

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and monocytes were removed by E rosetting and gamma globulin panning, respectively (E^-, FcR^- population). T cells were sheep E rosette$^+$ cells depleted of monocytes and Ia$^+$ cells by first panning on gamma globulin-coated dishes followed by panning on goat-anti-mouse dishes after incubation with an anti-Ia antibody. These T cells did not proliferate in response to Con A or anti-CD3 (OKT3) antibody. CD4$^+$ T cells were purified by coating E rosette$^+$ cells with a mixture of OKT8 (anti-CD8) and 9.3C9 (anti-class II) mAb followed by panning on 100-mm petri dishes (No. 1005, Falcon Labware, Oxnard, CA) previously coated with 120 μg of goat-anti-mouse Ig (Cappel Laboratories, Malvern, PA) in 10 ml PBS without calcium or magnesium (PD)[1]. The dishes were centrifuged twice at 35 g for 5 min at 4°C. Nonadherent cells were >95% CD4$^+$ lymphocytes and had <1% 3C10$^+$, Leu-16$^+$, Leu-2$^+$, or 9.3C9$^+$ cells.

The purity of T cells and accessory cells was monitored by staining with mAb analysis on a FACscan instrument (Becton Dickinson & Co., Mountain View, CA). Adherent cells were largely monocytes (>70%) by staining with 3C10 anti-CD14 mAb (indirect immunofluorescence with biotinylated horse anti-mouse Ig and FITC-avidin; Vector Laboratories, Inc., Burlingame, CA). The CD4$^+$ population was >95% pure as determined by staining with a combination of FITC-Leu-2 and phycoerythrin-Leu-3, (Becton Dickinson & Co.). The CD4$^+$ cells had no detectable MHC class II$^+$ cells or monocytes (CD14$^+$). Dendritic cells composed 3–5% of cells in the E^-, FcR^-, population and 25–50% of the Percoll-enriched fractions. Dendritic cells stained brightly with mAbs to class II MHC products (9.3C9, anti-HLA-DR; reference 4) but not with mAb to monocytes (3C10), NK cells (CD 16: 3G8, Leu-11b), B cells (CD19: Leu-16) or T cells (CD 3, 4, 8: Leu-2, 3, 4).

T Cell Clones. IL-2-dependent human T helper clones were grown as previously described (5, 6). The allospecific T helper clones were A-57 (DR-2 reactive) and 86 (DR-1 reactive). Both clones are CD3$^+$, CD4$^+$, and CD8$^-$ and have been shown to provide MHC-restricted helper activity for allogeneic B cells as measured by the induction of B cell proliferation and Ig secretion (5, 6). Before use, the T cell clones were washed out of FCS three times and then recultured in RPMI 1640 (Gibco Laboratories) containing 5% human AB$^+$ serum. These clones did not contain IL-1 as measured by immunofluorescence (see below).

Isolation of Dendritic-T Cell Clusters. To generate clusters of interacting T cells and dendritic cells, 1.5 × 10^6 T cells or CD4$^+$ cells were cultured with 0.3 × 10^6 partially enriched dendritic cells (E^-, FcR^- fraction) in 1.5 ml of complete medium in 15-ml round-bottomed plastic tubes (No. 2051; Falcon Labware). After 1–2 d of culture in the MLR or in the presence of 3 μg/ml Con A, the suspensions were gently collected and applied to Percoll gradients generated from a solution of three parts FCS, four parts isoperc, and 5.5 parts HBSS (7). Approximately 5–10 × 10^6 cells were applied to 12.5 ml gradients and centrifuged for 5 min at 4°C at 300 rpm. The clustered cells were found in the bottom portion of the gradient, and the nonclusters were found in the top 2 cm. The clusters were washed free of Percoll and then recultured or immediately cytospun onto glass slides for analysis of intracellular IL-1 (Shandon cytospin 2; Shandon Southern Instruments, Sewickley, PA). In some experiments, 30% serum gradients were used instead of Percoll gradients. Briefly, the mixture of cells was layered onto 5 ml of RPMI containing 30% AB$^+$ serum. The clusters pelleted after 15 min at 4°C.

Isolation of Antigen-specific Blasts. When dendritic-T cell clusters prepared as described above were cultured, antigen-specific T blasts developed and were released after 48–60 h (7). The blasts could be separated from residual clusters by reapplying the cell mixture to Percoll or serum gradients. The isolated blasts responded to 1–10 U/ml of rIL-2 but reverted to IL-2-unresponsive smaller cells if cultured without stimuli for 1–2 d (7).

Stimuli for IL-1 Production. Stimuli were usually applied for 24 h after which the culture fluid and cells were evaluated for IL-1 content (see below). The stimuli were: LPS, 20 ng to 20 μg/ml (Boivin preparation of *salmonella typhosa*; Difco Laboratories, Detroit, MI); PMA, 20 ng/ml (Sigma Chemical Co.); human rIFN-γ, 1.8 × 10^8 U/mg sp. act., 100 U/ml (gift of Hoffman-LaRoche, Nutley, NJ); Con A, 3 μg/ml (Pharmacia Fine Chemicals, Uppsala,

[1] *Abbreviations used in this paper:* GM-CSF, granulocyte/macrophage colony-stimulating factor; PD, PBS without magnesium or calcium.

Sweden); anti-CD3, OKT3, 25 ng/ml (Orthodiagnostic Systems, Raritan, NJ); Leu-4, 25 ng/ml (Becton Dickinson & Co., Mountain View, CA); rIL-4, 100 U/ml (DNAX, Palo Alto, CA); rGM-CSF; 5% cos7 supernatant and rIL-3; 5% cos7 supernatant (DNAX, kindly provided by Dr. T. Yokota); rTNF 25 ng/ml (kindly provided by Dr. Helen Vlassara, Rockefeller University, New York), rIL-6, 500 U/ml (DNAX). To evaluate the capacity of T cells to induce monocyte or dendritic cell IL-1, 5×10^4 enriched dendritic cells or 5×10^4 monocytes were mixed with 1.5×10^6 syngeneic or allogeneic blood T cells for 18–24 h. Syngeneic cultures were stimulated with Con A (3 μg/ml) or OKT3 (25 ng/ml). To evaluate the capacity of alloreactive T cell blasts or clones to turn on IL-1 in APC, they were cultured together at various ratios (see Results) for different time intervals, and the cells were analyzed as described below.

Identification of IL-1-producing Cells by Immunolabeling. Preimmune and immune rabbit sera generously provided by Drs. J. Schmidt and E. K. Bayne (8, 9) (Merck Institute of Research, Rahway, NJ) were used to stain cells as described (10). Briefly, cell suspensions were cytospun onto glass slides, fixed in paraformaldehyde, permeabilized in acetone, and stained with 1:200 dilution of rabbit serum followed by FITC-goat $(Fab')_2$ anti-rabbit IgG (Tago, Inc., Burlingame, CA). 100–200 cells from four different fields were counted to obtain the percentage of IL-1-producing cells. To identify monocytes, the cells were double-labeled with biotin-mAb (3C10 or anti-CD14), washed, and overlaid with phycoerythrin avidin (Becton Dickinson & Co.) or streptavidin-Texas Red (Amersham Corp., Arlington Heights, IL). This permitted quantitation of the percentage of monocytes synthesizing IL-1 (see Results).

D10.G4.1 ("D10") Bioassay for IL-1. The D10 line is a murine helper T clone kindly provided by Dr. C. A. Janeway (Yale University, New Haven, CT). The maintenance of the clone, as well as its direct proliferative response to IL-1, have been described (10, 11). Briefly, supernatants to be tested for IL-1 activity were added in graded doses of 50 to 0.15% vol/vol to $0.5–2 \times 10^4$ D10 cells in 0.1-ml medium in flat-microtest wells. [^{3}H]Thymidine was added at 4 μCi/ml from 60 to 72 h. The D10 line did not respond to the various stimuli that were used to induce IL-1 production from blood leukocytes including IL-4 and IL-6, while IL-2 was only minimally stimulatory (1,000-fold less sensitive than IL-1). The positive controls were: an IL-1-rich supernatant from LPS-activated peripheral blood monocytes; human rIL-1α, specific activities 5×10^6 U/mg (kindly supplied by Dr. P. Lomedico, Hoffman-LaRoche), human rIL-1β, specific activity 2.3×10^7 U/mg (kindly supplied by Dr. J. S. Schmidt, Merck Institute of Research). Standard curves relating volume of supernatant to the D10 response were used to determine IL-1 activity in U/ml where 1 U was defined as that giving half-maximal activity in the D10 assay. The D10 cell line responded half-maximally to the recombinant mouse and human IL-1 at a dose of 0.001–0.005 U/ml as defined by the thymocyte mitogenesis assay. Where activity was too low to be accurately determined from the standard curve, results are expressed as less than the lowest value measurable of the standard.

Thymocyte Proliferation Assay for IL-1. In some experiments, culture supernatants were tested with this assay. Briefly, 2×10^5 thymocytes from C3H/HeJ mice were cultured with 1.5 μg/ml of Con A and various dilutions of supernatant. Cultures were pulsed with 4 μCi/ml of [^{3}H]thymidine from 48 to 60 h. Results are expressed in cpm as averages of triplicates.

Assay for Detection of IL-1-inducing Factors. To determine if T cells after contact with accessory cells release IL-1-inducing factors, mixtures of T cells and APC were cocultured in upper or lower chambers of double-chambered dishes (0.4 μm, 6.5 mm Transwells; Costar, Cambridge, MA) with unstimulated accessory cells (monocytes) in the alternate chamber. Both populations were then analyzed for intracellular IL-1 production by immunofluorescence and for IL-1 secretion in medium by the D10 assay.

Capacity of Anti-IL-1 Antibody to Block T Cell Proliferation Assays. Responses to alloantigens were induced by adding graded doses of viable, irradiated (3,000 rad ^{137}Cs) accessory cells to 1.5×10^5 allogeneic T cells (MLR) or $2–4 \times 10^4$ allogeneic T blasts or cloned T cells in 6-mm flat wells (No. 3596, Costar) in medium with 5% AB$^+$ serum. In some experiments the accessory cells were not irradiated. For polyclonal mitogenesis, Con A (3 μg/ml) or OKT3 (25 ng/ml) was added to either 1.5×10^5 unseparated mononuclear cells or 1.5×10^5 T cells cultured with 3×10^4 enriched accessory cells. Proliferation was measured by the addition of 4 μCi/ml [^{3}H]thymidine from 48–60 h for Con A– and OKT3-stimulated responses, and

for growth of alloblasts and allogeneic clones. For primary MLRs, [^{3}H]thymidine was added from 120 to 136 h. Results are expressed in cpm as averages of triplicates. To test if neutralization occurred, rabbit polyclonal anti-IL-1 antibody (generously provided by Dr. R. Newton, du Pont de Nemours and Co., Glenolden, PA) was added to cultures at a final concentration of 50 μg/ml. At this dose, the antibody blocks ~20 U/ml of rIL-1α and rIL-1β in the thymocyte assay and comparable equivalent doses in the D10 assay. The antibody is an IgG fraction of immune sera that was prepared by immunizing a rabbit with human monocyte-derived IL-1α and IL-1β (12). The antibody does not significantly neutralize IL-2 or IL-6 bioactivity. To verify neutralization in cultures of APC and T cells, supernatants were assayed for IL-1 activity in the D10 bioassay as described below.

Results

Using a single cell immunolabeling technique to identify intracellular IL-1β we previously found that in blood and rheumatoid synovial effusions, the monocyte was the principal if not the only leukocyte synthesizing this cytokine in response to LPS (10). We proceeded to use this approach, together with the comparably sensitive D10 bioassay to look for IL-1 production during the interaction of monocytes and dendritic cells with T cells. In all cases we double labeled the leukocytes first with biotinylated anti-CD4 (antimonocyte) mAb followed by streptavidin Texas Red or phycoerythrin-avidin and then with anti-IL-1β plus FITC-goat anti-rabbit IgG so that we could discern monocytes from T cells in the culture.

IL-1 Production during Primary T Cell Proliferative Responses. When populations of blood mononuclear cells were stimulated with Con A or with OKT3 mAb for 18 h, IL-1 was evident in up to 25–33% of the monocytes (Table I). Lymphocytes were not labeled. If the blood cells were stimulated *after* separation into populations enriched in monocytes, T cells, and mixtures of dendritic cells and B lymphocytes, there was no IL-1 response to the mitogens although the monocytes responded to LPS (Table I). No IL-1$^+$ cells were seen in the mixtures of dendritic cells and B lymphocytes in response to LPS and Con A, but some IL-1 activity was detected in the medium (last two rows, Table I). In several other experiments, no IL-1 activity was detected in the medium of B cell–dendritic cell cultures stimulated with these agents. Other stimuli, PMA and IFN-γ, did not induce the human monocyte to synthesize IL-1 (Table I).

Allogeneic monocytes or dendritic cells were cultured with T cells in the MLR, but none of the cells were observed to contain IL-1 at 24 to 48 h. This was true even in clusters of interacting dendritic cells and lymphocytes (Fig. 1), which are the site in which the MLR is generated (7). At this stage T cell activation is already initiated since IL-2 is readily detected within the culture supernatants (7). Therefore it seems that IL-1 is not an early product in a primary, antigen-dependent response like the MLR.

Alloreactive T Cell Clones Induce IL-1 in HLA-DR-specific Monocytes, but not Dendritic Cells and B Lymphocytes. A different result was obtained when we examined the interaction of monocytes with previously stimulated T cells. A panel of human CD4$^+$ alloreactive T cell clones, restricted to specific HLA-DR molecules, was cocultured with different types of APC. DR-1$^+$ monocytes made IL-1 in response to the DR-1 specific clone 86, and DR-2$^+$ monocytes made IL-1 in response to the DR-2-specific clone A57 (Table II). IL-1 was also present in the culture medium by bioassay (Table II). In most but not all cases the percentage of IL-1$^+$ monocytes correlated with the

TABLE I

Monocytes Synthesize IL-1 in Response to LPS and Mitogen-stimulated T Cells

Population	Mitogen	IL-1$^+$ Monocytes	IL-1
		%	*U/ml*
Mononuclear cells	None	<0.1	<0.05
	LPS	88 ± 14	16
	Con A	26 ± 4	<0.05
	Leu-4	34 ± 11	<0.05
	OKT3	<0.1	10
	IFN-γ	<0.1	<0.05
Monocyte-enriched	None	<0.1	<0.05
	LPS	91 ± 6	67
	Con A	<0.1	<0.05
	Leu-4	<0.1	<0.05
	OKT3	<0.1	<0.05
	PMA	NT	<0.05
	IFN-γ	NT	<0.05
T cell-enriched	None	<0.1	<0.05
	LPS	<0.1	<0.05
	Con A	<0.1	<0.05
	OKT3	<0.1	<0.05
	IFN-γ	<0.1	<0.05
B and dendritic cell-enriched	None	<0.1	<0.05
	LPS	<0.1	5
	Con A	<0.1	14

Bulk mononuclear cells or fractions enriched in monocytes, T cells, or B plus dendritic cells, were stimulated for 18 h with the indicated stimuli. The percentage of monocytes synthesizing IL-1 was determined by the immunofluorescence assay, and the IL-1 released, by the D10 assay. NT, not tested.

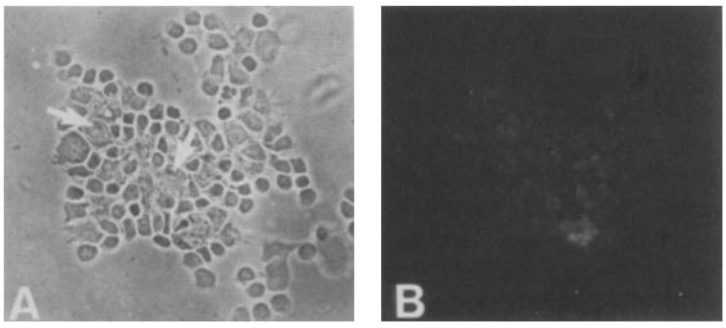

FIGURE 1. Aggregates of dendritic cells and allogeneic T lymphocytes, which are the site for T cell proliferation in the MLR, were isolated after 36 h of coculture by velocity sedimentation and were stained for IL-1. The clusters contain large dendritic cells (*A*, *arrows*) and smaller round lymphocytes. No staining is observed with anti-IL-1β antiserum (*B*) above the preimmune background. ×250.

TABLE II

Alloreactive T Cell Clones Induce IL-1 in HLA-DR-specific Monocytes

HLA-DR of APC	Stimulus	Number of cloned T cells	IL-1$^+$ monocytes	IL-1 released
			%	*U/ml*
DR-1	None		<0.1	0.1
	LPS		65 ± 0.5	54.0
	Clone A57	5×10^5	<0.1	0.1
	Clone 86	5×10^5	16 ± 6	7.7
		3×10^5	16 ± 4	5.2
		1×10^5	1 ± 1	2.5
		5×10^4	0.5 ± 1	0.2
DR-2	None		<0.1	0.04
	LPS		71 ± 2	97.00
	Clone 86	5×10^5	<0.1	<0.01
	Clone A57	5×10^5	13 ± 10	<0.01
		3×10^5	13 ± 2	10.00
		1×10^5	4 ± 0.5	<0.01
		5×10^4	3 ± 1	<0.01

Approximately 5×10^5 monocytes from donors bearing distinct HLA-DR specificities were cultured alone, with LPS, or with the indicated numbers of two alloreactive T cell clones. Clone 86 is specific for DR-1, and clone A57 for DR-2. The percentage of monocytes synthesizing IL-1 was determined at 24 h using the immunolabeling assay, and the IL-1 bioactivity in the culture medium using the D10 proliferation assay. The monocyte-enriched populations were 70–83% CD14$^+$ in the different experiments.

secreted IL-1 activity. The IL-1$^+$ cells were monocytes and not T cells since they were double labeled with anti-CD14. The frequency of IL-1$^+$ monocytes was proportional to the T cell dose, with optimal responses involving 15–30% of monocytes at a 1:1 monocyte/T cell ratio (Table II). No further increase in the proportion of IL-1$^+$ monocytes was noted with a fourfold excess of T cells (not shown).

The time course of IL-1 production was examined over a 24-h period. At optimal clone-to-monocyte ratios, IL-1 was detected as early as 6 h and peaked at 24 h (Table III), which is similar to the kinetics observed with LPS (10). The number of IL-1$^+$ monocytes declined after 24 h (not shown).

Mixtures of dendritic cells and B cells were prepared by depleting blood mononuclear cells of T cells and monocytes by rosetting with sheep erythrocytes and panning on IgG-coated plates, respectively. <1% of these APC stained with anti-CD14 monocyte mAb. 3–5% were dendritic cells in cell size and shape and by strong staining with anti-MHC class II but lack of staining with Leu-12 and Leu-16 anti-B cell reagents. The mixtures of B cells and dendritic cells induced strong proliferation of the T cell clone, but neither type of APC nor the T cells made IL-1 even when LPS was added (Table IV). These findings indicate that monocytes are the main source of IL-1 during HLA-DR-restricted interactions with T cell clones and suggest that IL-1 is not essential for antigen specific proliferation by the T cell clone.

Alloreactive T Cell Blasts Induce IL-1 in MHC-specific Monocytes, but not other Leukocytes. To assess if the findings with T cell clones were applicable to freshly sensitized, alloreactive T cells, the latter were isolated from a primary MLR and rechallenged with HLA-DR-specific and nonspecific APC. Coculture of the T blasts

TABLE III

Kinetics of T Cell-mediated IL-1 Induction in Monocytes

Exp.	Culture time	HLA-DR of monocytes	IL-1$^+$ monocytes after stimulation with: None	LPS	Clone 86 (α-DR-1)	Clone A57 (α-DR-2)
	h				%	
A	0	DR-1	<0.1	1.6 (.2)	<0.1	<0.1
	24	DR-1	<0.1	52 (2)	53 (1)	<0.1
B	0	DR-2	<0.1	1.3 (2)	<0.1	0.6 (1)
	2	DR-2	<0.1	<0.1	<0.1	<0.1
	6	DR-2	<0.1	39 (1)	<0.1	9.0 (3)
	24	DR-2	<0.1	73 (5)	<0.1	8.0 (2)

Monocyte-enriched populations, from HLA-DR1 (Exp. A) or HLA-DR2 (Exp. B) individuals, were cultured with LPS or with clones 86 and A57 for the indicated times. The percentage of CD14$^+$ cells synthesizing IL-1 (standard deviations) was determined by the immunolabeling assay.

with monocytes at a 1:1 ratio for 12 h was sufficient to induce IL-1 in 10–25% of class II-specific but not nonspecific or syngeneic monocytes (Table V). In one instance, alloreactive T blasts (anti-DR-2,7) did not induce significant IL-1 synthesis in MHC-specific monocytes (DR-2,7). This may be due to the fact that alloblasts are a more heterogeneous population than cloned T cell lines. Alternatively, the dose-response requirements may have been different for this particular preparation of T cells.

The T cells had to be activated to induce IL-1 since no induction of cytokine was observed in mixtures of monocytes and fresh, allogeneic T cells (see above). As with the studies of T cell clones (see above), dendritic cells and B cells did not make IL-1 in cultures set up in parallel with the monocyte-T blast mixtures (data not shown).

Mechanism of IL-1 Induction in Monocytes by T Cells. The mechanism whereby acti-

TABLE IV

Monocytes, but not Dendritic Cells and B Cells Make IL-1 in Response to T Cell Clones

Stimulus	IL-1 induction in different APC (percent IL-1$^+$) DR-1$^+$ Monocytes	DR-4$^+$ Monocytes	DR-1$^+$ B + DC	DR-4$^+$ B + DC
None	<0.1	<0.1	<0.1	<0.1
LPS	76 ± 9	91 ± 12	<0.1	<0.1
Clone 86, α-DR-1	13 ± 0.2	<0.1	<0.1	<0.1
	DNA synthesis per culture (cpm [^{3}H]TdR)			
None	217	217	407	223
Clone 86, α-DR-1	8,525	213	13,909	165

Monocytes and enriched populations of B cells and dendritic cells (B + DC) were cultured with the indicated stimuli. The percentage of APC synthesizing IL-1 at 20 h was calculated using the immunolabeling assay. DNA synthesis was measured by adding [^{3}H]TdR from 48 to 60 h. The cpm for the T cell clone cultured in the absence of APC was 158 cpm.

TABLE V

Alloreactive T Cell Blasts Induce IL-1 in HLA-specific Monocytes

Exp.	Donor of monocytes	Stimulus	IL-1	IL-1$^+$ monocytes
			U/ml	%
A	DR-1,5	None	<0.1	<0.1
		LPS	297	74 ± 18
		α-DR1,5 T cell	49	18 ± 7
		α-DR-2,7 T cell	<0.1	<0.1
	DR-2,7	None	<0.1	<0.1
		LPS	281	92 ± 12
		α-DR-1,5 T cell	<0.1	<0.1
		α-DR-2,7 T cell	<0.1	2 ± 0.3
	DR-7	None	10	2 ± 0.2
		LPS	329	59 ± 7
		α-DR-1,5 T cell	65	6 ± 0.8
		α-DR-2,7 T cell	29	11 ± 0.7
B	DR-2,7	None		<0.1
		LPS		73 ± 10
		α-DR-2,7 T cell		26 ± 4
		α-DR-5 T cell		<0.1
	DR-5	None		<0.1
		LPS	NT	93 ± 9
		α-DR-2,7 T cell		<0.1
		α-DR-5 T cell		15 ± 5
	DR-4,10	None		<0.1
		LPS		94 ± 0.4
		α-DR-2,7 T cell		<0.1
		α-DR-5 T cell		<0.1

Equal numbers of CD4$^+$ T cell blasts were cultured with monocytes from different donors. α-DR-1,5 blasts were raised by culturing accessory cells from JM with T cells from JS (DR-2,7), and α-DR-2,7 blasts were developed from cocultures of JM T cells and JS accessory cells. JM and JS are disparate at all loci in the HLA complex, while donor PSF (DR7) appears to share determinants with both JS and JM, since blasts generated against either JS or JM proliferated to PSF. Supernatants from the cultures of blasts and APC were tested in the D10 assay, and the IL-1 released was determined by regression analysis against a standard of rIL-1β. The percentage of monocytes synthesizing IL-1 was determined by immunolabeling with an α-IL-1β antibody and double labeling with α-CD14, 3C10 mAb. The percentage of CD14$^+$ cells in each fraction was: JM, 87; JS, 73; PSF, 78.

vated T cells induce IL-1 in monocytes was analyzed. Two possibilities were considered: the clone or lymphoblast could trigger the monocyte by direct cell–cell interaction, or the APC or T cell could release an IL-1-inducing cytokine, as suggested by data from studies with murine T cell clones (13, 14). Monocytes were cultured in media that had been conditioned in three different APC-T cell cocultures: clone 86 and DR-1$^+$ monocytes; alloblasts with MHC-specific monocytes; or the primary MLR between dendritic cells and T cells. Concentrations of supernatant in the range of 25–50% did not induce IL-1 (not shown). There was no suppressive factor in the supernatants since the addition of LPS induced IL-1 (not shown). Several specific recombinant cytokines did not induce IL-1 in monocytes including: IL-4 (100 U/ml),

TNF/cachectin (25 ng/ml), IL-6 (500 U/ml), IL-3 (5% cos7 supernatant), GM-CSF (5% cos7 supernatant), and IFN-γ (100 U/ml).

To further exclude a role for IL-1-inducing cytokines, two chamber experiments were performed. Alloreactive T cells and MHC-specific monocytes or enriched dendritic cells were cultured in one well which was separated from third-party fresh monocytes by a 0.4-μm filter. In three of four experiments, no IL-1 was detected (not shown). In a fourth experiment, a small percentage of monocytes (3%) synthesized IL-1 by immunolabeling, compared with 12% synthesized in the cocultures of alloblasts and MHC-specific monocytes. This was not due to carryover of endotoxin, since <5 pg/ml of endotoxin was detectable in the culture supernatants. Of interest was the finding that a 30% dilution of supernatant from the alloblast and MHC-specific monocyte cultures also induced 4% of fresh third-party monocytes to produce IL-1. Thus a more potent pathway for IL-1 induction in monocytes seems to be through direct *MHC-specific* contact rather than via release of inducing factors.

Induction of IL-1 in HLA-DR Nonspecific Monocytes in Cocultures of T Cell Clones and HLA-DR-specific Dendritic Cells. A remarkable result was obtained when we cocultured DR-1 dendritic cells, DR-1-specific clone 86, and DR-3,4 nonspecific monocytes. The latter were found to synthesize IL-1, and to a comparable extent to DR-1 monocytes cultured with clone 86 (Table VI, Fig. 2). DR-3,4 monocytes, however, would not synthesize IL-1 when cultured with clone 86 in the absence of DR-1 dendritic cells (2nd and 4th groups in Table VI).

Identical results were obtained with alloreactive T blasts (not shown). Coculture of alloreactive blasts, MHC-specific dendritic cells, and nonspecific "bystander" mono-

TABLE VI

IL-1 Induction in HLA-DR-nonspecific Monocytes by an Alloreactive T Clone

APC population	Stimulus	IL-1-bearing monocytes
		%
DR-1 monocytes	None	<0.1
	LPS	77 ± 5
	Clone 86, α-DR-1	34 ± 18
DR-3,4 monocytes	None	<0.1
	LPS	78 ± 13
	Clone 86, α-DR-1	<0.1
DR-1 B cell plus DC	None	<0.1
	LPS	1 ± 0.6
	Clone 86, α-DR-1	0.3 ± 0.2
	DR-3,4 monocytes	<0.1
	Clone 86 + DR-3,4 monocytes	18 ± 0.5
DR-3,4 B cell plus DC	None	<0.1
	LPS	<0.1
	Clone 86, α-DR-1	<0.1
	DR-1 monocytes	<0.1
	Clone 86 + DR-1 monocytes	25 ± 2

APC from DR-1 or DR-3,4 donors were cultured in the absence of stimuli, or with LPS, clone 86, and/or monocytes from either donor. After 20 h the cells were analyzed for IL-1 production by the immunolabeling assay.

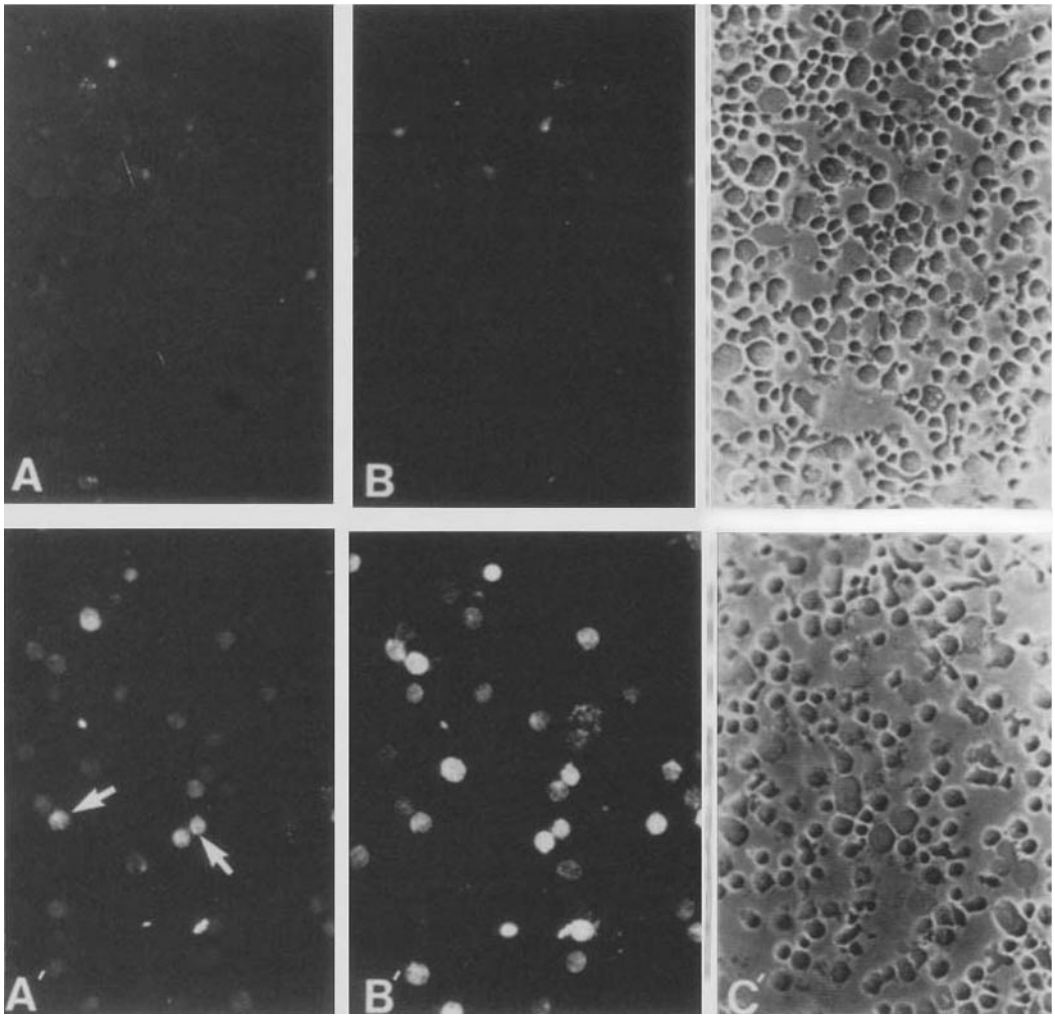

FIGURE 2. DR-specific dendritic cells and alloreactive T cell clone induce IL-1 in non-DR-specific monocytes. Enriched dendritic cells, monocytes, and T cell clone were cultured for 24 h and stained with biotinylated 3C10 (anti-CD14) and streptavidin-Texas Red, followed by anti-IL-1 antisera and FITC-conjugated goat anti-rabbit IgG. (*A–C*) DR-1 DC cultured with clone 86 (DR-1-reactive). (*A′–C′*) DR-1 dendritic cells cultured with clone 86 and DR-5 monocytes. (*A*, *A′*) FITC stain, (*B*, *B′*) streptavidin stain; (*C*, *C′*) phase contrast of the same respective fields. IL-1$^+$ monocytes are indicated by white arrows (*A′*) and represent ~20% of the total monocyte population. ×250.

cytes led to IL-1 synthesis in the latter. The MHC nonspecific monocytes could be third-party or syngeneic to the T blasts. However, as noted above, if dendritic cells and T blasts were placed in one chamber separated from monocytes by a 0.4-μm filter in another, little or no IL-1 induction occurred.

These results suggest that when T cells are being stimulated by dendritic cells, some MHC-nonrestricted mechanism leads to induction of IL-1 by monocytes, presumably by cell contact rather than an IL-1-inducing factor.

TABLE VII

Anti-IL-1 Antibody Does Not Block the Proliferation of an Alloreactive T Cell Clone to Monocytes, B Cells, or Dendritic Cells

Allogenic Stimulator	Proliferation of T cell clone ([^{3}H]TdR incorporation)			
	No clone	Clone 86, no Ig	Clone 86, anti-IL-1	Clone 86, control Ig
			cpm	
DR-1 monocytes	1,484	30,627	29,568	26,270
		(42)	(<0.0001)	(41)
DR-1 monocytes	815	52,733	55,201	56,604
		(83)	(0.8)	(83)
DR-7 monocytes	785	878	893	280
		(5.4)	(<0.0001)	(6.0)
DR-1 B cells + DC	1,349	65,689	63,082	36,533
		(1.8)	(<0.0001)	(6.3)
DR-1 B cells + DC	1,806	30,565	23,619	29,397
		(17)	(10)	(23)
DR-7 B cells + DC	3,224	3,431	901	1,670
		(16)	(6)	(18)

Proliferation of clone 86 alone was 989 cpm. The anti-IL-1 antibody and rabbit IgG were added at 50 μg/ml. Parentheses denote amount of IL-1 released in culture supernatant expressed as U/ml of activity in the thymocyte co-stimulation assay. Two different DR-1$^+$ donors were used in the above experiment.

TABLE VIII

Anti-IL-1 Does Not Block the Proliferation of Alloreactive T Blasts to Antigen-bearing Monocytes, B Cells, or Dendritic Cells

Exp.	Added as APC	HLA-DR to which T blasts are primed	Antibody added	Response to APC: HLA-DR 1,5	Response to APC: HLA-DR 2,7
				cpm × 10^{-3}	
A	Monocytes	DR-1,5	None	71.0	8.7
			Control Ig	60.3	13.0
			α-IL-1	58.5	10.0
	DC + B cells	DR-1,5	None	87.5	7.4
			Control Ig	73.3	5.3
			α-IL-1	69.8	7.8
				HLA-DR-1,DQwl	HLA-DR 2,7
B	Monocytes	DR-2,7	None	2.8 (<0.01)	18.6 (58)
			Control Ig	3.8	17.0
			α-IL-1	6.8 (<0.01)	28.3 (<0.01)
		DR-1, DQwl	None	48.8 (147)	3.7 (<0.01)
			Control Ig	35.6	2.9
			α-IL-1	49.3 (<0.01)	11.0 (<0.01)

CD4$^+$ T cells were induced to become blasts in an MLR to dendritic cells (DC) (see Materials and Methods). The blasts were rechallenged with the indicated syngeneic or allogeneic APC and antibodies; [^{3}H]TdR uptake was measured at 72–90 h. The cell doses were Exp. A: 10^5 blasts and either 10^5 monocytes or 3×10^4 DC + B cells; Exp. B: 2×10^4 blasts and 1.5×10^5 monocytes. In Exp. B we also cultured 5×10^5 blasts for 12 h with 10^6 monocytes; only the HLA-specific monocytes made IL-1 (45% IL-1$^+$ monocytes) by the immunolabeling assay (not shown). The supernatants were collected and assayed for IL-1 activity in the D10 assay. The units/milliliter IL-1 are shown in parentheses. The proliferation of the T blasts without APC ranged from 0.5 to 2.2×10^3 cpm.

Significance of IL-1 Production in the T Cell Proliferative Response. Additional experiments were performed to assess the role of IL-1 in T cell proliferation, given the evidence that this cytokine was produced when monocytes rather than dendritic cells were the APC. First we added a polyclonal anti-IL-1 α/β antibody using nonimmune rabbit Ig as control. To ensure that IL-1 was being produced and neutralized in these cultures, supernatants from the same microtiter wells were assayed for IL-1 in the thymocyte comitogenesis assay. As shown in Table VII, IL-1 was produced in cocultures of clone 86 (anti-DR1) and DR1 monocytes but not DR7 monocytes. This IL-1 was neutralized by anti-IL-1 antibody but not control Ig. Some thymocyte costimulatory activity was present in dendritic-T cell cocultures, but this was probably due to IL-2 since it was not neutralized by anti-IL-1. When either monocytes or dendritic cells were the APC, anti-IL-1 did not neutralize the proliferative response of the T cell clone (Table VII).

Similar results were obtained with freshly sensitized T blasts, that is, anti-IL-1 did not block the proliferative response to allogeneic monocytes or dendritic-T cell mixtures (Table VIII). The IL-1 that was present in the monocyte-T blast cocultures was neutralized with anti-IL-1 in the D10 assay. Furthermore, neutralizing anti-IL-1 did not block the primary MLR or polyclonal mitogenesis to Con A, OKT3, or PHA when monocytes or dendritic cells were the accessory cells (data not shown).

Discussion

There is evidence that IL-1 is produced by APC during their interaction with murine T cells (13, 14), and that IL-1 enhances T cell proliferative responses (2, 15, 16). However, the production and significance of IL-1 in cultures of freshly isolated leukocytes, as opposed to long-term cell lines, has not been well characterized. There is recent evidence that murine T cell clones make IL-1 (17), so that the contribution of T cells in fresh leukocyte populations needs to be considered. The single cell immunolabeling approach of Bayne et al. (8) has allowed us to study IL-1 production in human cells and to make the following findings.

Antigen-dependent, MHC-restricted Induction of IL-1. IL-1 was detected during the MLR to HLA-DR specific stimulators, but there were two requirements for optimal production. One was that the T cell had to be preactivated, since alloreactive T blasts and T cell clones induced IL-1 but unprimed T cells did not. The second was that monocytes made IL-1, but dendritic cells and B cells did not. Furthermore, the induction in monocytes was MHC restricted. Dendritic cells did not make IL-1 even when clustered with T cells during the first 48 h of an MLR (Fig. 1). At this stage T cell activation is already initiated since IL-2 can be detected in culture supernatants (7). In no instance did we detect IL-1 in the T cell. Monocytes also produced IL-1 in T cell-dependent responses to lectin and to anti-CD3 mAb (Table I), but it is not clear if the effective T cells were unprimed lymphocytes or if they had been primed in situ. It is of interest that even though all monocytes were heavily rosetted with T cells in the presence of lectin or anti-CD3 (not shown), only a minority made IL-1 (Table I).

Together these observations suggest that IL-1 production is primarily a product of the efferent rather than afferent limb of a T cell-mediated immune response. Comparable conclusions have been reached in the murine system, although a single cell

assay was not available. There, a cDNA probe for IL-1α mRNA was used on populations of macrophages and alloreactive T blasts that were separated and probed after their antigen-dependent interaction (1). Only the monocytes were induced to express IL-1α mRNA. In contrast to LPS, which induced the majority of monocytes to synthesize IL-1, alloreactive T cells induced IL-1 in 10–30% of MHC-specific monocytes. This difference could be due to the degree of physical interaction occurring between T cells and monocytes in culture. However, even with a fourfold excess of T cells, no increase in the frequency of IL-1$^+$ monocytes was detected. Alternatively, only discrete subsets of monocytes or T cells may be involved in the induction of IL-1.

An MHC-unrestricted Pathway for IL-1 Production. The observation that dendritic cells did not make IL-1 allowed us to look for MHC unrestricted pathways for monocyte IL-1 production. The first observation was that IL-1-inducing factors could not be definitively identified in two-chamber protocols. When T cell clones or blasts were undergoing intense proliferative responses to MHC-specific dendritic cells in one chamber, and MHC nonspecific monocytes were placed in the other, the monocytes did not make IL-1. However, if the three cell types were cultured *together* in one vessel, monocyte IL-1 was induced. IL-1 was not detected in control experiments in which the dendritic cells were MHC nonspecific, or either dendritic cells or T cells were omitted.

It is conceivable that IL-1-inducing factors are released but that these can only act across short distances rather than across a filter. Alternatively, dendritic cells and/or T cells release factors that alter the monocyte so that it physically interacts and responds to T blasts in an antigen-independent or MHC-unrestricted fashion. For example, Dustin et al. (18) have shown that IFN-γ can upregulate ICAM-1 on keratinocytes and that the latter then bind T blasts by an LFA-1-dependent mechanism. This sort of antigen-independent mechanism might greatly amplify IL-1 production during an antigen-initiated immune response.

Function of IL-1 in T Cell Proliferation. The fact that dendritic cells stimulate T cells but do not make IL-1 when cultured with LPS (10, Table I) or responding T cells (Fig. 1, Tables IV and VI) suggests that IL-1 is not essential for T cell activation in man or in mice (1, 11). These experiments were extended using a neutralizing anti-IL-1 antibody that did not block dendritic cell–mediated responses to alloantigen or Con A (Tables VII and VIII). We were unable to block T cell proliferative responses to antigen-bearing monocytes even though the latter were making IL-1, and the IL-1 in the medium was neutralized (Tables VII and VIII). Therefore, unless the anti-IL-1 antibody does not gain access to zones of intercellular contact, one must question the longstanding view that IL-1 is an important T cell stimulant, which to our knowledge has not been supported by experiments in which the proliferation of primary T cell populations has been blocked by anti-IL-1.

In the mouse, IL-1 can be essential for (19), or might enhance (20), the growth of IL-4-dependent T cell lines. It is of interest that one of the T cell clones we used, clone 86, makes IL-4 but not IL-2 (21). Supernatants from clone:APC cocultures fail to induce growth of the IL-2 dependent line CTLL but can enhance CD23 (Fcϵ receptor) expression on B cells (21), a property determined to be dependent upon IL-4 (22). Clone 86 is as efficient as fresh, alloreactive T blasts in inducing IL-1,

but it still proliferates in the presence of neutralizing anti-IL-1. Further studies of other IL-4-producing cells will help evaluate the generality of the role of IL-1 in inducing growth of IL-4-producing T cell clones and primary populations.

Another role for IL-1 could be to induce IL-6, as from fibroblasts (23), and IL-6 would then act as a growth factor for T cells (24–26). We are studying the production of IL-6 by human APC, but as yet we have not detected a role for IL-1 in inducing IL-6 in either dendritic cells or monocytes, in contrast to diploid fibroblast cell lines.

Yet another contribution of IL-1 to T cell proliferation is to enhance the function and production of dendritic cells, which can then act on the T cell in an IL-1-independent fashion (2, 27). In our experience, IL-1-pulsed human dendritic cells were two- to threefold more active accessory cells for both the MLR and lectin responses. By this pathway, exogenous IL-1 could indeed amplify T cell proliferation without acting directly as a lymphocyte-activating factor.

Summary

We have studied the control and significance of IL-1 production in human leukocyte cultures during accessory cell–dependent, T lymphocyte mitogenesis using sensitive bioassays and immunolabeling techniques. In primary antigen-dependent systems like the MLR, IL-1 production was not detected in accessory cells (monocytes, dendritic cells) or T cells, suggesting that it is not an early product in these responses. However, monocytes could be induced to make IL-1 after interacting with sensitized antigen-specific T cells. Both alloreactive T cell clones or freshly prepared lymphoblasts induced IL-1 provided the monocytes carried the HLA-DR antigens to which the T cells were initially sensitized. Even in these circumstances, dendritic cells and B cells failed to make IL-1.

The mechanism whereby activated T cells induce IL-1 in monocytes was explored. Supernatants from cocultures of monocytes and T cells or several recombinant cytokines induced little or no IL-1. A more potent antigen independent pathway of IL-1 induction was identified. IL-1 could be induced in third-party HLA-DR nonspecific monocytes in cocultures of alloreactive T cell clones or blasts and HLA-DR-specific dendritic cells. The induction was factor independent since dendritic cells and T blasts placed in a chamber separate from third-party monocytes by a semipermeable membrane did not induce monocyte IL-1. These results suggest that a cell contact mechanism rather than an IL-1-inducing factor leads to IL-1 production.

The role of IL-1 in T cell proliferation was tested with a polyclonal anti-IL-1 antibody. The antibody failed to block the proliferation of primary T cells, or alloreactive T cell clones and blasts stimulated with HLA-specific monocytes or dendritic cells, even though IL-1 in the medium was neutralized.

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