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Immunology

Antibodies against membrane interleukin 1α activate accessory cells to stimulate proliferation of T lymphocytes

(B lymphocytes/monocytes/interleukin 2)

ELSIE M. EUGUI* AND SUSAN J. ALMQUIST

Department of Immunology, Syntex Research, S3-10, 3401 Hillview Avenue, Palo Alto, CA 94304

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ABSTRACT **Some monoclonal antibodies (mAbs) against interleukin (IL) 1α have been found to activate antigen-presenting cells (APC, human peripheral blood monocytes and B lymphocytes), so that unstimulated T lymphocytes cultured with them are induced to proliferate and secrete IL-2. Control mAbs of the same isotypes and mAbs against IL-1β do not activate APC. In the absence of APC, mAbs against IL-1α do not induce proliferation of T lymphocytes. Mitomycin C-treated activated APC still induce T-cell proliferation. Proliferation of T lymphocytes cannot be induced by culture supernatants and requires contact with APC activated by mAbs against IL-1α. The observations imply that surface membrane IL-1α can function as a triggering molecule on APC, which could play an important role in the initiation of immune responses by T lymphocytes.**

Although much information has accumulated about the structure of T-lymphocyte receptors (TCR) for antigen and the role of major histocompatibility glycoproteins (MHC class I and class II) in antigen presentation, the mechanism by which T lymphocytes are induced to respond to antigens is not well understood. In particular, the role of a form of interleukin (IL) 1 associated with the surface membrane of APC is controversial (1–3).

The IL-1α and IL-1β genes encode primary translation products of relative molecular mass of ≈31–33 kDa (4, 5): The 31-kDa form of IL-1α binds to receptors and is biologically active, whereas the 31-kDa form of IL-1β does not (6); both are cleaved to proteins of ≈17.5 kDa that are biologically active (6). The two forms of human IL-1 have only ≈26% amino acid sequence correspondence, although the base sequence correspondence of the genes encoding them is greater (45%). Nevertheless, IL-1α and IL-1β bind with nearly equal affinity to a receptor that has recently been cloned, expressed, and sequenced (7). Hence, it is not surprising that IL-1α and IL-1β share many biological activities, including induction of IL-2 production by certain T-lymphocyte cell lines and costimulation of thymocytes (6).

Nevertheless, there are indications that the fates of the two molecules after translation differ. IL-1β is more rapidly secreted from activated human monocytes than IL-1α (8, 9). Monoclonal antibodies (mAbs) against IL-1α bind to lipopolysaccharide (LPS)-activated human peripheral blood monocytes, as shown by flow cytometry, whereas mAbs against IL-1β do not (10). These observations suggest that IL-1α preferentially becomes associated with the surface membrane of the cells in which it is produced in such a way that some determinants of IL-1α are accessible to extracellular antibodies.

Interest in the possible biological role of a surface membrane form of IL-1 has been independently generated by observations that murine peritoneal macrophages and B lymphocytes lightly fixed can stimulate the proliferation of T-cell lines and clones (1, 11). IL-1 bioactivity of fixed, LPS-activated human monocytes was inhibited by polyclonal antibodies to IL-1α but not those binding to IL-1β (10). However, it has recently been shown that LPS-activated, paraformaldehyde-treated macrophages continuously release IL-1α (2, 3), which has been taken as evidence against the existence of a membrane-associated form of IL-1α (2).

We now report that some mAbs against IL-1α, but not those against IL-1β, activate APC so that they induce proliferation of and IL-2 production by unstimulated cocultured T lymphocytes. These observations provide further evidence that surface membrane IL-1α not only exists but plays a role in activating accessory cells for T lymphocyte-mediated immune responses.

MATERIALS AND METHODS

mAbs. All mAbs against IL-1α and IL-1β used were raised in our laboratory. The mAb against recombinant human IL-1β (rHuIL-1β) have been fully described (12). The four mAbs used in these studies (H-6, H-21, H-34, and H-67) block IL-1β biological activity *in vitro* and react with rHuIL-1β in immunoassays, but not with rHuIL-1α. The mAbs against homogeneous rHuIL-1α (Immunex, Seattle) were also raised in our laboratory by using a similar protocol (13). These mAbs were also purified as described (12), and their isotypes are either IgG2a or IgG1. The blocking activity of these antibodies in IL-1α-induced proliferation assays is described below. The mAbs against IL-1α do not cross-react with IL-1β in biological assays (see below) or immunoassays. The mAbs against IL-1α and IL-1β have affinities for their respective IL-1 $>7 \times 10^9$ liter/mol and do not bind IL-2, IL-4, IL-6, tumor necrosis factor α, or basic fibroblast growth factor.

Anti-Leu 12 (Becton Dickinson), a mouse IgG1 mAb specific for an antigen expressed on human B lymphocytes (CD19, 95 kDa), was used as an additional control. Before use in culture, azide was removed from this mAb by passing it through an Ultrafree-MC filter unit, 30,000 NMWL polysulfone PTTK membrane (Millipore).

Bioassays for IL-1. A C3H/HeJ mouse thymocyte comitogenic assay and a human fibroblast proliferation assay were used as described (12). rHuIL-1α or rHuIL-1β (500 pg/ml) were standards in both assays.

Isolation and Culture of Human Peripheral Blood Mononuclear Cells (PBMC). PBMC were separated from heparinized blood of normal volunteers by centrifugation in Ficoll-Paque

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Abbreviations: mAb, monoclonal antibody; IL, interleukin; APC, antigen-presenting cells; PBMC, peripheral blood mononuclear cells; LPS, lipopolysaccharide; TCR, T-lymphocyte receptor; MHC, major histocompatibility complex; smIL-1 and smIL-1α, surface-membrane IL-1 and IL-1α, respectively; rHuIL-1α and rHuIL-1β, recombinant human IL-1α and IL-1β, respectively.

*To whom reprint requests should be addressed.

(Pharmacia). After being washed, cells were resuspended in RPMI 1640 medium/penicillin at 50 units/ml/streptomycin at 50 μg/ml/L-glutamine at 2 mM/25 mM Hepes buffer/5% (vol/vol) heat-inactivated fetal bovine serum. This medium, designated complete RPMI, was used to culture all human cells. Cells were cultured in 96-well plates at 2×10^5 cells per well, with and without mAb. Each antibody dilution was tested in quadruplicate. Cultures were incubated for 72 hr at 37°C in 5% CO_2 and 100% humidity. A pulse of [^{3}H]thymidine (dThd) was added for the last 16 hr, and the cells were collected and processed for radioactivity measure as described (12).

Separation of Adherent Cells, T Cells, and B Cells. PBMC in suspension in the RPMI medium described above were seeded at various densities in 96-well plates. After 1-hr incubation at 37°C in 5% CO_2, the nonadherent cells were removed, and adherent cells were washed three times and reconstituted with complete RPMI medium. The proportion of nonspecific esterase-positive cells in the plates was >95%.

T cells were isolated from PBMC after depletion of adherent cells by incubation in plastic dishes (4×10^6 cells per well, 6-well plates) for 1 hr at 37°C in 5% CO_2. Nonadherent cells were collected and incubated with sheep erythrocytes (Microbiological Media) treated with 2-aminoethylisothiouronium bromide (Aldrich). T-rosetting lymphocytes (E^+ lymphocytes) were separated by Ficoll-Paque density centrifugation. Sheep erythrocytes were lysed by incubation at 37°C for 10–15 min in autologous plasma. After washing, E^+ cells were further depleted from monocytes by passage through a Sephadex G-10 column (Pharmacia), ≈7-ml vol in a 10-ml plastic syringe. The number of cells loaded onto each column did not exceed 40×10^6. The resulting T-cell population contained <0.1% esterase-positive monocytes. The T-cells were resuspended in complete RPMI and cultured at 2×10^5 cells per well with mAb or were added unstimulated to other cell populations in coculture experiments. B-cell-enriched populations were obtained from nonadherent, nonrosetting human PBMC after passage through a Sephadex G-10 column. The final B-cell-enriched population contained <2% nonspecific esterase-positive monocytes and <15% of T cells by staining with fluorescein isothiocyanate-labeled anti-Leu 5 mAb (Becton Dickinson) and by fluorescein-activated cell sorter analysis.

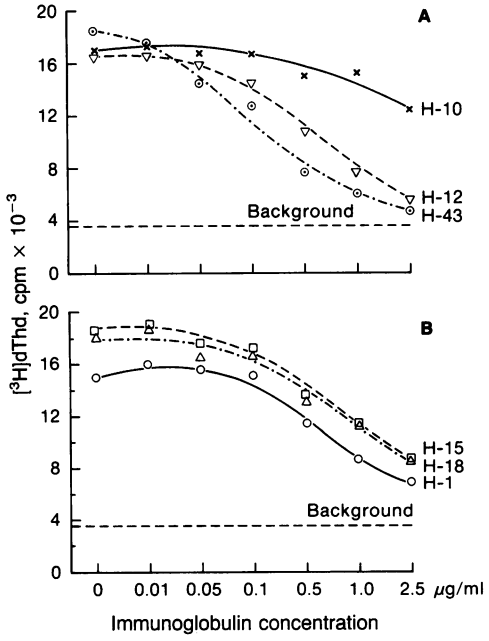

FIG. 1. Inhibition of IL-1α-induced fibroblast proliferation by anti-IL-1α mAb. mAbs (*A* and *B*) together with rHuIL-1α were added to fibroblasts (5×10^3 per well), and cultures were incubated 48 hr before adding [^{3}H]dThd (0.5 μCi per well, 18 hr). Each point represents the mean value for quadruplicate wells.

Table 1. Some mAbs against IL-1α but not against IL-1β stimulate proliferation of human PBMC

mAb	Specificity	Concentration, μg/ml	[^{3}H]dThd uptake by PBMC, cpm × 10^{-3} (mean ± SD) Exp 1	Exp 2
None	—	—	0.4 ± 0.07	0.7 ± 0.05
P 1.17	(IgG2a)*	20	0.8 ± 0.54	0.4 ± 0.10
P3X63Ag8	(IgG1)*	20		0.2 ± 0.05
H-1	IL-1α	10	4.5 ± 3.75	6.8 ± 0.22
		20	14.9 ± 5.56	11.0 ± 3.28
H-10	IL-1α	10	0.3 ± 0.04	0.3 ± 0.09
		20	0.4 ± 0.09	0.2 ± 0.10
H-12	IL-1α	10	0.5 ± 0.09	0.2 ± 0.02
		20	0.6 ± 0.09	0.2 ± 0.06
H-15	IL-1α	10	0.7 ± 0.24	2.7 ± 0.05
		20	3.4 ± 0.89	5.2 ± 2.7
H-18	IL-1α	10	3.6 ± 4.30	6.2 ± 0.38
		20	5.3 ± 2.68	8.8 ± 0.26
H-43	IL-1α	10	9.3 ± 4.82	
		20	23.2 ± 9.38	
H-6	IL-1β	10		0.5 ± 0.10
		20		0.9 ± 0.20
H-21	IL-1β	10		0.2 ± 0.05
		20		0.1 ± 0.03
H-34	IL-1β	10		0.6 ± 0.12
		20		1.7 ± 0.24
H-67	IL-1β	10	0.6 ± 0.33	0.5 ± 0.01
		20	0.4 ± 0.08	0.9 ± 0.33
Leu 12	CD19	1		0.5 ± 0.20
		10		0.4 ± 0.12

*Control mouse antibodies. Exp, experiment.

Coculture Experiments. B cells or adherent cells, were incubated with mAb for 24 hr as indicated above. The cells were washed three times. In some experiments, these cells were treated with mitomycin C (Sigma) for 30 min at 37°C and washed three times. Autologous, nonstimulated T cells were added at 2×10^5 cells per well, and cultures were incubated for an additional 72-hr period under identical conditions, and pulsed with [^{3}H]dThd (0.5 μCi per well; 1 Ci = 37 GBq) overnight before harvesting.

IL-2 Evaluation in Supernatants from mAb-Stimulated PBMC. Human PBMC were incubated as described above (2×10^5 cells per well) with or without mAbs against IL-1α. Supernatants were collected after 24, 48, and 72 hr, and IL-2 activity was evaluated using a standard assay (14). As control, PBMC from the same donor were stimulated with Con A (Sigma, 2.5 μg/ml), and the supernatants were collected at the same intervals.

Table 2. Total PBMC, but not purified T lymphocytes, proliferate in response to anti-IL-1α mAb stimulation

mAb	Specificity	Concentration, μg/ml	[^{3}H]dThd uptake by PBMC, mean cpm ± SD PBMC	T lymphocytes
Exp 1				
H-43	IL-1α	0	703 ± 574	78 ± 3
		10	5,501 ± 3,263	259 ± 155
		20	4,316 ± 1,761	223 ± 98
Exp 2				
H-43	IL-1α	0	210 ± 104	194 ± 23
		20	6,769 ± 1,105	226 ± 235
		40	10,199 ± 970	219 ± 59

Table 3. Activation of human PBMC by mAb H-43 against IL-1α

Cells	mAb H-43, μg/ml	Lymphocytes added after 24 hr	[^{3}H]dThd uptake,*cpm
PBMC			
2×10^5	0		74 ± 9
2×10^5	10		45,819 ± 4,141
2×10^5	40		59,290 ± 8,116
T lymphocyte			
2×10^{-5}	0		156 ± 76
2×10^{-5}	10		184 ± 53
2×10^{-5}	40		696 ± 47
B lymphocyte			
2×10^{-5}	0		1,375 ± 42
2×10^{-5}	10		1,460 ± 174
2×10^{-5}	40		2,954 ± 322
Monocyte†			
4×10^{-5}	0	B	644 ± 95
4×10^{-5}	10	B	1,101 ± 91
4×10^{-5}	40	B	1,214 ± 4
4×10^{-5}	0	T	108 ± 41
4×10^{-5}	10	T	2,113 ± 403
4×10^{-5}	40	T	7,760 ± 1,736
2×10^{-5}	0	T	135 ± 53
2×10^{-5}	10	T	1,256 ± 128
2×10^{-5}	40	T	9,273 ± 1,784
1×10^{-5}	0	T	92 ± 17
1×10^{-5}	10	T	2,578 ± 1,179
1×10^{-5}	40	T	7,702 ± 2,057
B-lymphocyte			
2×10^{-5}	0	T	1,934 ± 468
2×10^{-5}	10	T	11,799 ± 2,062
2×10^{-5}	40	T	32,932 ± 3,438
1×10^{-5}	0	T	808 ± 122
1×10^{-5}	10	T	5,021 ± 1,077
1×10^{-5}	40	T	13,569 ± 1,225

*Each value represents mean ± SD of quadruplicate determinations.
†Number of monocytes indicated is the number of PBMC originally seeded per well before removing nonadherent lymphocytes. Cells were stimulated with mAb H-43 during the whole experiment, except for cocultures, when monocytes and B cells were stimulated with mAb H-43 only for the first 24 hr.

RESULTS

Blocking IL-1α Biological Activity by mAbs. Six mAbs against IL-1α were tested for their ability to block IL-1-induced human fibroblast proliferation (Fig. 1). mAbs H-34 and H-12 at 1 μg/ml (6×10^{-9} M) almost completely blocked the activity of IL-1α at 500 pg/ml (3×10^{-11} M). With exception of mAb H-10, all the other mAbs block IL-1α-induced fibroblast proliferation. The same mAbs also inhibit IL-1α-induced thymocyte proliferation. mAbs H-43 and H-12 were the most potent, requiring <1 μg/ml to almost completely block the proliferation induced by IL-1α at 500 pg/ml, whereas mAb H-10 had only a partial inhibitory effect at the highest concentration tested (2.5 μg/ml) (data not shown). The other three mAbs were of intermediate potency (data not shown). None of the mAbs significantly inhibited thymocyte or fibroblast proliferation induced by IL-1β (data not shown).

Induction of Human PBMC Proliferation by mAbs. Table 1 shows that four of six mAbs against IL-1α added to unstimulated human PBMCs stimulated incorporation of [^{3}H]dThd into DNA. The most potent mAb was H-43, which also most strongly inhibited IL-1α bioactivity. mAbs H-1, H-15, and H-18 also stimulated DNA synthesis significantly, although less potently than mAb H-43. Two other mAbs against IL-1α (H-10 and H-12) did not stimulate DNA synthesis. Four mAbs, binding with high affinity to nonoverlapping epitopes of IL-1β (12), and control mAb of the same isotypes as the active mAb against IL-1α did not stimulate [^{3}H]dThd incorporation. Another mAb reacting with a different molecule expressed on the surface of B cells, anti-Leu 12, did not stimulate cell proliferation either (Table 1). This result excludes the possibility that any mAb bound to the surface of APC can activate T cells by means of the Fc portion of the immunoglobulin.

Cell Types Activated by mAb to IL-1α. To define which cells in PBMC were involved in the response to the anti-IL-1α mAb, T cells were incubated with mAb H-43, and proliferation was measured after 3 days. As shown in Table 2, T cells did not proliferate in the absence of accessory cells. In addition, mAbs added to cultures of monocytes did not stimulate [^{3}H]dThd incorporation and, when added to cultures enriched in B lymphocytes, stimulated DNA synthesis only twofold, presumably because of a small number of residual T cells in the preparation (Table 3). These observations suggested that mAbs against IL-1α can activate accessory cells (B lymphocytes or monocytes), so that when unstimulated T lymphocytes are added to them, DNA synthesis is induced.

Cocultures of mAb-Stimulated Accessory Cells with Unstimulated T Cells. To test further the conclusion that IL-1α in accessory cells triggers their function, B lymphocytes and monocytes were separately incubated with mAb H-43 for 24 hr and washed; in the absence of the mAb unstimulated autologous T lymphocytes were cocultured with the preactivated B lymphocytes or monocytes for 3 days and [^{3}H]dThd incorporation was measured. Table 3 shows that potent stimulation of DNA synthesis was seen in the cocultures with preactivated B lymphocytes, and less potent but still highly significant stimulation was seen in the cocultures with preactivated monocytes. In contrast to previously described coculture systems, preactivation of T lymphocytes by lectins, antibodies against CD3, or other manipulations was not required. Table 3 shows that increasing the proportion of B lymphocytes increases T-lymphocyte stimulation, whereas with monocytes a plateau is reached. Either the B lymphocytes are more efficient in the accessory function described or the monocytes are also releasing a suppressive factor.

To further define that proliferating cells are T lymphocytes, B-cell-enriched populations were incubated with mAb H-43 for 24 hr and treated with mitomycin C before unstimulated T cells were added to the cultures. As shown in Table 4, T-cell responses were not affected by blocking proliferation of the activated accessory cells in this way. In addition, Leu 12^+ B cells sorted with the fluorescence-activated cell sorter (FACStar PLUS, Becton Dickinson) had comparable activity to the B-cell-enriched population after incubation with mAb H-43 (data not shown).

T-Cell Stimulation Is Not Mediated by a Soluble Factor. The next question addressed was whether the accessory cell stimulation of T lymphocytes is mediated by a soluble factor

Table 4. Mitomycin C treatment of mAb H-43-activated B cells does not alter the response of cocultured T lymphocytes

Cultured cells	Treatment* Mit.	Treatment* T cells added after 24 hr	[^{3}H]dThd uptake, cpm
PBMC no. 2	−	−	6,580 ± 800
B cells no. 1	+	−	429 ± 47
	+	+	13,992 ± 794
B cells no. 2	+	−	318 ± 86
	+	+	14,142 ± 277

*B cells were incubated with mAb H-43 at 40 μg/ml for 24 hr, treated with mitomycin C (Mit), and washed before autologous T cells were added.

or factors released into the culture medium. B lymphocytes and monocytes were cultured with mAb H-43 for 24 hr, and supernatants were added to autologous unstimulated T lymphocytes. Supernatants of activated B lymphocytes or monocytes did not stimulate the T lymphocytes even in dilutions as low as 1:1 (data not shown). When mAb-activated monocytes or B lymphocytes were separated from T lymphocytes by a 0.4 μM polycarbonate membrane in a Transwell chamber (Costar), no proliferation of T lymphocytes was observed, whereas control PBMC in such chambers responded in the expected fashion (data not shown). These observations demonstrate that the major stimulatory mechanism activated in accessory cells by antibodies against IL-1α requires contact with responder T-lymphocytes.

IL-2 Production by PBMC Stimulated with mAb H-43. To assess whether the T-cell proliferative response to mAb H-43 is mediated by IL-2, PBMC were stimulated with this mAb, and supernatants were collected at 24, 48, and 72 hr. The same PBMC were also stimulated with Con A, and supernatants were collected at identical intervals. The mAb H-43 stimulation resulted in abundant production of IL-2, with maximum activity in the 48-hr supernatants; after Con A stimulation, maximal IL-2 production was at 24 hr (Fig. 2). By using anti-IL-2 receptor mAb conjugated with fluorescein isothiocyanate (Becton Dickinson) and flow cytometry analysis, an increase in the percentage of T lymphocytes expressing IL-2 receptor was observed in the mAb H-43-stimulated PBMC (data not shown). These observations confirm that the cells responding to mAb H-43 stimulation in PBMC are T lymphocytes activated to express IL-2 receptor and produce IL-2.

Time Course of the PBMC Response to mAb H-43. Because the IL-2 activity in supernatants from PBMC stimulated with mAb H-43 was maximum at 48 hr and still detectable 72 hr after initiation of the cultures, we tested proliferation at longer intervals. As shown in Fig. 3, PBMC stimulated with mAb H-43 (10 μg/ml) induced proliferation $\approx$3 times higher on days 4 and 5 than on day 3.

DISCUSSION

The use of mAbs to define cell-surface molecules triggering proliferation of lymphocytes and cytokine production is well established. For example, anti-CD3 stimulates proliferation of T lymphocytes in the presence of monocytes (15, 16), and mAb recognizing different epitopes of the CD2 molecule, used in combination, induces proliferation (17). Anti-CD28 increases proliferation in the presence of submitogenic concentrations of lectin mitogens or anti-CD3; it regulates production of many T-lymphocyte-derived cytokines (18).

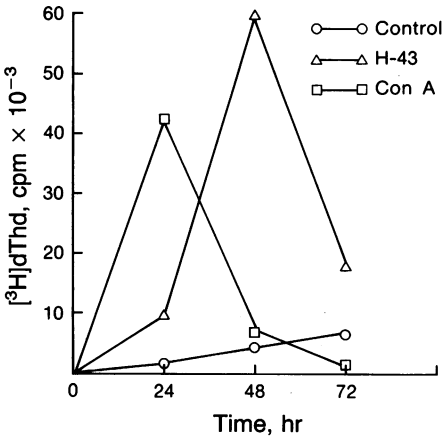

FIG. 2. IL-2 activity in the supernatant of PBMC stimulated with mAb H-43 or Con A. Supernatants were collected after 24-, 48-, and 72-hr incubation, and IL-2 was measured (14).

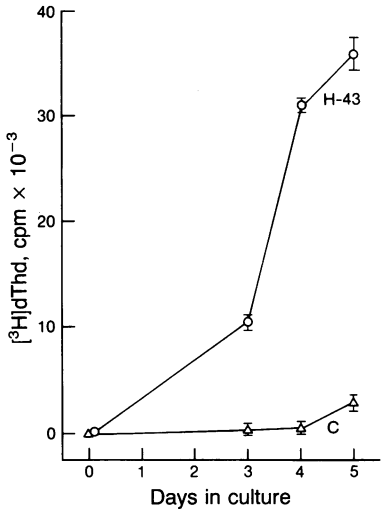

FIG. 3. Time course of the PBMC response to mAb H-43 stimulation. PBMC were cultured for 3, 4, and 5 days as described with or without (C) mAb H-43 at 10 μg/ml.

These observations suggest that interaction of surface-membrane IL-1α (smIL-1α) in APC (B lymphocytes and monocytes) with antibodies and, presumably, other ligands can activate mechanisms in those cells so that they can stimulate proliferation of T lymphocytes on contact. Contact of activated APC with responding T lymphocytes is necessary because separation of the cells by a polycarbonate membrane prevents the response. IL-1β is not demonstrable on the surface of APC, and mAbs against IL-1β do not activate them. A mAb binding to another surface membrane protein on APC (CD19) does not induce T-cell proliferation, indicating that the Fc portion of immunoglobulin bound to APC alone is unlikely to be a mediator in this activation. Although LPS stimulates mouse lymphocyte proliferation, it does not have this effect on human lymphocytes. When several LPS concentrations were used to stimulate PBMC, no response was seen (data not shown). Hence, endotoxin contamination of the mAb used (in any case, below detectable levels) cannot explain our findings. Likewise, IL-1α added to PBMC cultures does not induce proliferation (data not shown) and is mitogenic for thymocytes only in the presence of lectins. Supernatants from APC stimulated with mAb to IL-1α do not induce T-cell proliferation either. Hence, released IL-1α or any other soluble mediator produced by APC is probably not responsible for triggering the T-cell activation.

The role of cells of the monocyte/macrophage lineage in antigen presentation to T lymphocytes has been well documented (19). More recently evidence has been obtained that B lymphocytes can also present antigens to helper T lymphocytes—at least in peripheral lymphoid tissues (20, 21). Two properties of B lymphocytes and cells of the monocyte/macrophage lineage are important in their function as presenters of antigen to T lymphocytes. (*i*) One is expression of class II MHC glycoproteins, which bind antigens (22), thereby facilitating interaction with the TCR (23); cells bearing class II molecules also interact preferentially with T cells expressing CD4, whereas cells bearing class I molecules do so with T cells expressing CD8 (24). (*ii*) The second property of activated B lymphocytes and monocytes, which we investigated, is their capacity to stimulate proliferation of the T lymphocytes that they contact. This mechanism could induce clonal expansion of T lymphocytes with TCR for the antigen on the surface of activated accessory cells. *In vivo* those T lymphocytes presumably remain in contact with accessory cells longer than do other T cells because of interaction of the

TCR with antigen and MHC glycoproteins and of the latter with CD4 or CD8; hence the proliferation of antigen-specific cells would be preferentially stimulated. B cells binding antigen are more efficient than other B cells in eliciting specific immune responses (20).

Both class II MHC glycoprotein expression and smIL-1α expression can be regulated in B lymphocytes and monocytes. For example, interferon γ increases class II MHC glycoprotein expression in macrophages (25), and IL-4 increases it in B lymphocytes (26). LPS increases smIL-1α expression in monocytes and smIL-1 bioactivity in macrophages (1, 27). Contact with T cells or supernatants of activated T cells increase smIL-1 expression in B lymphocytes (1) and macrophages (28), whereas IL-2 stimulates the production of IL-1α and IL-1β by human mononuclear cells (9). Hence, antigenic stimulation may be an autocatalytic process, with B lymphocytes activating T cells whereas products of the latter, in turn, augment the efficiency of accessory cell function. When smIL-1α is expressed, antibodies binding to these molecules trigger accessory cell function to stimulate T-lymphocyte proliferation and IL-2 production, as we described. Presumably other ligands, for example the IL-1 receptor expressed on the surface of T lymphocytes and many other cell types (7), can bind smIL-1α and similarly trigger accessory cell function.

Many questions remain unanswered, including the reason why IL-1α preferentially associates with the plasma membrane. Does it bind to a surface membrane-associated protein, in a manner analogous to binding of TCR α and β chains to the T3 complex (29), which increases expression of the TCR at the cell surface? Is smIL-1α 31–33 kDa or 17.5 kDa, and which epitopes are accessible to extracellular ligands? Numerof *et al.* (9) report that in activated human monocytes IL-1α remains cell-associated in a 33-kDa form, whereas most of the IL-1β is secreted in an 18-kDa form. In murine macrophages surface labeling shows IL-1α in two forms, one 33-kDa and the other 80-kDa (27). The former, likely to be the primary product of the IL-1α gene, is seen after LPS stimulation; the 80-kDa form may be IL-1α complexed with another membrane protein and is present in unstimulated macrophages. Conceivably such a complex is also present in human monocytes and B lymphocytes and is activated by mAbs reacting with IL-1α sites accessible on the cell surface. The availability of mAbs that either activate or do not activate accessory cells will facilitate epitope definition.

What is the mechanism by which activated accessory cells stimulate T-cell proliferation? Activated accessory cells express proteins that facilitate interactions with and proliferation of T lymphocytes—e.g., intercellular adhesion molecule 1 that binds LFA-1 antigen on T lymphocytes (30). We are currently investigating the expression of these and other activation antigens on B lymphocytes exposed to mAb against IL-1α. Although these questions are important, the observations now presented already show a major difference between IL-1α and IL-1β: the former can function as a regulatory molecule in the cell in which it is synthesized, whereas IL-1β is secreted and activates cells in the vicinity.

There is an analogy with immunoglobulins that are used by B lymphocytes as both membrane receptors for antigens and as secretory products. The membrane-associated and secretory forms of μ heavy chains are specified by mRNAs transcribed from a single gene containing alternative secretory and membrane exons at its 3′ end (31). Resting B cells express principally mRNA for the membrane form of μ, but when immunoglobulin secretion is stimulated by LPS (32) or T-cell growth or differentiation factors (33), production of mRNA for the secreted form is increased. Our findings show that smIL-1α is expressed in unstimulated B lymphocytes and monocytes. Activation of the latter markedly increases IL-1β gene expression (6), and we have found this also for the IL-1α gene. Because our observations imply that smIL-1α is active within the cell membrane and does not have to be secreted, the criticisms of Minnich–Carruth and colleagues (2) become irrelevant. The concept that a cytokine can function as a membrane receptor is provocative. Whether this function is true of other cytokines remains to be seen.

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