LYMPHOKINE ENHANCES THE EXPRESSION AND SYNTHESIS OF Ia ANTIGENS ON CULTURED MOUSE PERITONEAL MACROPHAGES*

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I-region-associated (Ia) antigens are more readily detected on mouse dendritic cells (DC)[1] and B lymphocytes than on peritoneal and spleen macrophages (Mϕ) from the same animals (1). In contrast, expression of Mϕ Ia increases in mice infected with live mycobacteria (Bacille Calmette-Guérin) (2, 3). We therefore asked if Mϕ Ia could be influenced by lymphokine (LK) released by T cells mediating anti-microbial immunity. We have chosen to study the effects of LK from *Trypanosoma cruzi*-immune mouse spleen cells. This source of LK is a potent activator of Mϕ microbicidal, secretory, and tumoricidal capacities (4–6). The effects of LK on the Mϕ surface were monitored using radioiodinated monoclonal antibodies directed to Ia and four other plasma membrane antigens. We report that addition of immune LK selectively enhances expression and synthesis of Mϕ Ia. Populations that <5% Ia positive can be converted to >95% positive. However, these homogeneous populations of Ia-bearing Mϕ do not stimulate the primary mixed leukocyte reaction (1° MLR).

Materials and Methods

Mice. Male and female B6D2F$_1$, C57BL/6, and DBA/2 (Trudeau Institute, Saranac Lake, N. Y.) and CBA/J (The Jackson Laboratory, Bar Harbor, Maine) mice were used, generally at 6–8 wk of age.

Cells. Peritoneal Mϕ were obtained from mice primed 4 d previously with 1 ml proteose peptone intraperitoneally. 2 h after explantation, nonadherent cells were removed by washing, and the adherent monolayer maintained in Dulbecco's medium supplemented with 10% heat-inactivated fetal calf serum (D10; both reagents from Grand Island Biological Co., Grand Island, N. Y.) and antibiotics. The adherent cells were >95% Mϕ because they were phagocytic for opsonized erythrocytes and expressed the Mac-1 surface antigen (7), which is abundant on mouse Mϕ but weak or absent on mouse B cells and DC. (Nussenzweig et al. Manuscript in preparation.) Adherent cells were maintained on 13-mm diameter glass cover slips (Gold Seal, No. 3550). DC were obtained from mouse spleen as previously described (1, 3). Proteins on coverslips that bore Mϕ were measured with a Lowry assay using lysozyme as standard.

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[1] *Abbreviations used in this paper:* D10, Dulbecco's medium supplemented with 10% fetal calf serum; DC, dendritic cell(s); HKT, heat-killed *Trypanosoma cruzi*; LK, lymphokines(s); Mϕ, macrophage(s); MHC, major histocompatibility complex; MLR, mixed leukocyte reaction(s); 1° MLR, primary MLR.

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LK. Spleen cells were dissociated from groups of three to four CBA/J mice that were unprimed or were infected with *T. cruzi* 2–3 wk earlier as described (5, 6). 10^8 spleen cells were cultured for 48 h in 6.5 ml Dulbecco's medium supplemented with 2% fetal calf serum, antibiotics, and 5×10^{-5} 2-mercaptoethanol in 100-mm tissue culture dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). Immune LK refers to medium from *T. cruzi*-immune cells boosted with 6×10^6 heat-killed *T. cruzi* (HKT) in vitro; control LK usually refers to nonimmune cells boosted with HKT. CBA (Ia^k) mice were used to generate LK because this strain does not react with the anti-$Ia^{b,d}$ reagent used in these experiments (see below). Therefore LK-induced changes on $Ia^{b,d}$ cells are Mϕ rather than LK derived. LK was added to peritoneal Mϕ at a final concentration of 1–30% vol:vol and changed daily. Six different preparations of immune, and four preparations of control LK, were tested with similar effects. To determine if LK production required T cells, we treated spleen cells with a monoclonal rat anti-mouse Thy-1 (our clone B5-3) and complement (Rabbit LOTOX; Accurate Chemical & Scientific Corp., Hicksville, N. Y.) before culture with specific antigen.

Monoclonal Antibodies. Five monoclonal rat anti-mouse reagents were used, all produced by hybridizing immune rat spleen with the mouse myeloma P3U-1, kindly supplied by Dr. M. Scharff, Albert Einstein College of Medicine, Bronx, N. Y. Clones were picked on soft agar, and antibody-rich ascites formed during growth in irradiated mice. Ig rich in monoclonal antibody was purified by passage over DEAE-cellulose. Three non-MHC reagents were derived by Dr. J. Unkeless, The Rockefeller University, New York, with rat spleen cells immune to the mouse Mϕ cell line J774. The three clones that react with all spleen and peritoneal Mϕ were: 1.21 J a clone that reacts with mouse Mϕ and granulocytes but not with most lymphocytes and DC and appears to be similar by immunoprecipitation analysis to clone Mac-1 described by Springer et al. (7) (8); 2.6, another clone that recognizes a 21,000-mol wt polypeptide found on mouse Mϕ (8); and 24G2 a clone that recognizes the IgG_{2b}, or trypsin-resistant, Fc receptor (9). An Fab fragment of clone 24G2 antibody, produced by papain cleavage, was used (9). The anti-major histocompatibility complex (MHC) reagents were produced in rats immunized with live BALB/c (H-2d) DC intravenously. Binding studies with iodinated monoclonal antibodies were performed on spleen cells of appropriate MHC congenic and recombinant strains. Clone B21-2 seems to be an anti $I\text{-}A^{b,d}$, and clone B25-1 an anti $H\text{-}2D^d$ reagent (Table I). Clone B21-2 immunoprecipitates typical class II polypeptides (10) and competes in sequential immunoprecipitation protocols with anti-Ia 8 (anti-$I\text{-}A^{b,d}$), but not anti-Ia 7 (anti-$I\text{-}E^{d,k}$), alloantisera obtained from the Research Resources Branch, National Institutes of Health, Bethesda, Md. Clone B25-1 precipitates typical class I (45 and 13 mol wt) polypeptides (10).

Binding of Monoclonal Antibodies. Each of the monoclonal reagents was iodinated with carrier-free $Na[^{125}I]$ (New England Nuclear, Boston, Mass.; or Amersham/Searle, Arlington Heights, Ill.) to a level of 1–2 μCi/μg and stored as 20–50 μg/ml solutions in phosphate-buffered saline to which sodium azide (0.02%) and 1 mg/ml bovine serum albumin were added. Chloramine T or iodogen (Pierce Chemical Co., Rockford, Ill.) was used for iodination. Quantitative binding studies were performed on ice using cells adherent to 13-mm glass cover slips. Duplicate cover slips were exposed to 0.2 ml antibody at 0.2–1.0 μg/ml culture medium with 0.02% sodium azide. 1 μg/ml is at or close to the level required to saturate most binding sites for all antibodies. Equilibrium was reached within 30–60 min, after which the cover slips were rinsed through three successive changes of medium and fixed in 1.25% glutaraldehyde. The cover slips were counted (Autogamma 5220; Packard Instrument Co., Inc., Downers Grove, Ill.) and, when necessary, processed further for light microscope radioautography as described (3). Controls for the specificity of binding in both quantitative and radioautographic assays included: (*a*) binding of the monoclonal was inhibited close to the background level by a 20-fold excess of unlabeled specific antibody; (*b*) binding was not inhibited if saturating levels of the anti-Fc receptor reagent, clone 24G2, were included during the binding assay; and (*c*) in the case of anti-MHC reagents, binding above the no cell background was not seen with Mϕ from the inappropriate haplotype, e.g., CBA. In all cases, background binding on cover slips without cells was <5–10% of specific binding. We suspect that the monoclonal antibodies have access to the Mϕ surfaces facing the culture medium and the culture vessel, because Dr. J. Michl, The Rockefeller University, (Personal communication.) has demonstrated that concanavalin A binds diffusely to the plasma membrane at 4°C.

TABLE I

Binding of Monoclonal Anti-MHC Antibodies to Mouse Spleen

Mouse strain	Major histocompatibility complex							Clone	Antibody bound by 2.5×10^5 spleen cells			
	K	I-A	I-B	I-J	I-E	I-C	D		1.0 μg/ml	0.5 μg/ml	0.25 μg/ml	0.125 μg/ml
									cpm			
No cells				—					562	302	189	125
B10.BR	k	k	k	k	k	k	k		415	196	116	110
B10.A	k	k	k	k	k	d	d		338	202	132	98
B10.D2	d	d	d	d	d	d	d		17,615	11,345	6,095	3,032
C57BL/6	b	b	b	b	b	b	b	B21-2 (anti-I-$A^{b,d}$)	10,440	7,167	4,538	2,828
B10.A(5R)	b	b	b	k	k	d	d		11,404	7,397	4,056	2,450
B10.A(4R)	k	k	b	b	b	b	b		500	221	151	102
C3H	k	k	k	k	k	k	k		408	210	154	90
C3H.OH	d	d	d	d	d	d	k		26,542	12,359	7,084	3,662
No cells				—						122	106	52
B10.BR	k	k	k	k	k	k	k			100	74	79
B10.A	k	k	k	k	k	d	d			1,234	1,059	505
B10.D2	d	d	d	d	d	d	d			1,045	939	495
C57BL/6	b	b	b	b	b	b	b	B25-1 (anti-H-$2D^d$)		153	102	95
B10.A(5R)	b	b	b	k	k	d	d			1,294	858	523
B10.A(4R)	k	k	b	b	b	b	b			107	84	66
C3H	k	k	k	k	k	k	k			94	92	65
C3H.OH	d	d	d	d	d	d	k			90	84	66

2.5×10^5 spleen cells from several mouse strains were spun onto 96-well microtest dishes (Costar, Data Packaging, Cambridge, Mass.) coated with poly-L-lysine (40 μg/ml). The dishes were rinsed in cold medium containing 5% serum and then 0.2 ml of ^{125}I-monoclonal antibody was added in various doses. Both clone B21-2 and B25-1 had a 4.5×10^6 cpm/μg sp act. Equilibrium was reached within 1 h. The cells were washed in medium and lysed in 0.1 N NaOH before counting. Data are the means of triplicate determinations with SD of 5–20%. Strains bearing Ia-reactive cells in binding studies were 50–70% Ia-positive by cytotoxicity assays. Binding studies of this sort were performed at least three times on each strain with similar results. C57BL/10 mice (not shown here) behaved similarly to C57BL/6. Mice were provided by Dr. Perrin White, The Rockefeller University. The polypeptides precipitated by clones B25-1 and B21-2 from radiolabeled spleen cells are typical class I and II MHC polypeptides, respectively (10).

Biosynthesis of Plasma Membrane Antigens. Adherent peritoneal Mϕ were maintained on 60-mm glass Petri dishes in D10 medium with or without immune or control LK. At 54 h, the medium was changed to methionine-free medium supplemented with 60 μCi/ml of [^{35}S]-methionine (1017 Ci/mM; New England Nuclear), 10% dialyzed serum, with or without LK. After an additional 14 h, the monolayers were washed, lysed in 1.2 ml lysis buffer (8), and clarified by centrifugation at 45,000 *g* for 15 min at 4°C. An aliquot (2×10^6 cpm) was immunoprecipitated for 1 h at 4°C with a cocktail that contained 1 μgm each of monoclonal antibodies 1.21 J, B25-1, and 2.6. The immunoprecipitates were retrieved on protein A-Sepharose coated with immunoselected rabbit anti-rat Ig (N. L. Cappell Labs, Cochranville, Pa.). Another aliquot (10×10^6 cpm) was immunoprecipitated for 1 h on B21-2 coupled to cyanogen bromide-activated Sepharose 4B. The Sepharose beads were washed and solubilized as previously described (10), and the antigens separated by 4–11% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis. To quantitate the amount of labeled antigen, individual bands were excised, solubilized in 20% perchloric acid-20% hydrogen peroxide, and counted in the presence of Hydrofluor (National Diagnostics Inc., Parsippany, N. J.).

Mixed Leukocyte Reactions (MLR). B6D2F$_1$ irradiated stimulator Mϕ or DC (1,500 rad, Cs^{137} source), were added to 16-mm tissue culture wells that contained 4.5×10^6 nylon-wool-filtered responder DBA/2 spleen cells in 1 ml of RPMI-1640 medium supplemented with 1% responder

mouse serum or 1% fetal calf serum. The cultures were maintained for 3–4 d, the cells were harvested, and then [^{3}H]thymidine uptakes were measured in fresh medium as previously described (11). In some cultures, indomethacin (1 μg/ml, Sigma Chemical Co., St. Louis, Mo.) was added to block synthesis of inhibitory Mϕ prostaglandins (3, 12).

Results

Effect of LK on Ia Antigens of Cultured Mϕ. Expression of Ia was followed for 6 d in cultured peritoneal Mϕ. In one-half of the cultures, the D10 medium was supplemented with 25% vol:vol immune LK, i.e., culture medium from HKT-boosted *T. cruzi*-immune spleen cells. At each time point, radioiodinated clone B21-2 antibody was used to quantitate (Fig. 1) and visualize (Fig. 2) Mϕ Ia. Throughout the experiments, ≥90% of the cells remained attached and were viable in a trypan blue assay.

At the onset of culture, binding of ^{125}I-anti-Ia was readily detectable (Fig. 1) and distributed in a heterogeneous fashion (Fig. 2A). A subpopulation (5–30%) of the cells were strongly positive. After a day of culture in D10, or D10 with immune LK, binding of anti-Ia dropped dramatically (Fig. 1) and <5% of the cells were Ia positive by autoradiography (Fig. 2B). No cell loss was evident during the first day of culture, and the levels of other plasma membrane antigens were not decreased (see below). Because the initial Ia positive cells were Mϕ (Materials and Methods), we conclude that expression of Ia is short-lived on most peritoneal Mϕ in culture.

Ia remained at low levels on Mϕ cultured without LK (Fig. 1, ●—●), and where detectable, was always restricted to a very small subpopulation of cells. But in the continued presence of LK, Ia was re-expressed, reaching a plateau level by day 3 (Fig. 1, Δ—Δ). At saturation, Mϕ expressed 1 × 10^5–2 × 10^5 binding sites/cell. By radioautography, it was determined that LK induced virtually all cells to express Ia (Fig. 2C). Enhanced expression of Ia was dose-dependent, reaching one-half-maximal levels at 1% vol:vol LK and a plateau at 10% vol:vol (data not shown). Binding of ^{125}I-anti-Ia was specific, because CBA (Ia^k) Mϕ exposed to LK did not bind the anti $Ia^{b,d}$ reagent above the no cell control (e.g., Fig. 2D).

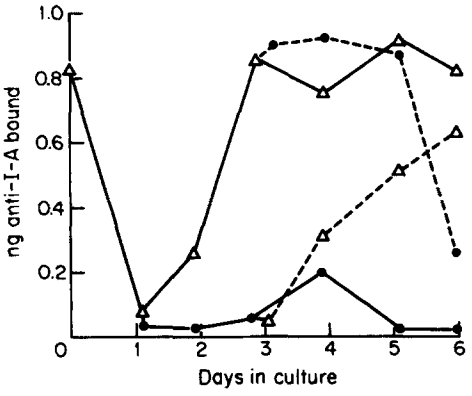

FIG. 1. Effects of immune LK on the expression of Ia. Peritoneal Mϕ were maintained for 6 d in D10 medium (●—●) or immune LK (25% vol:vol, Δ—Δ), all media were changed daily. In one additional set of cultures, the D10 medium was replaced by immune LK beginning at day 3 (Δ---Δ), whereas in another, the LK was removed by washing and replaced by D10 at day 3 (●---●). Similar data to those illustrated from day 0–3 were obtained in seven consecutive experiments, whereas day 3–6 data were obtained in 3 consecutive experiments.

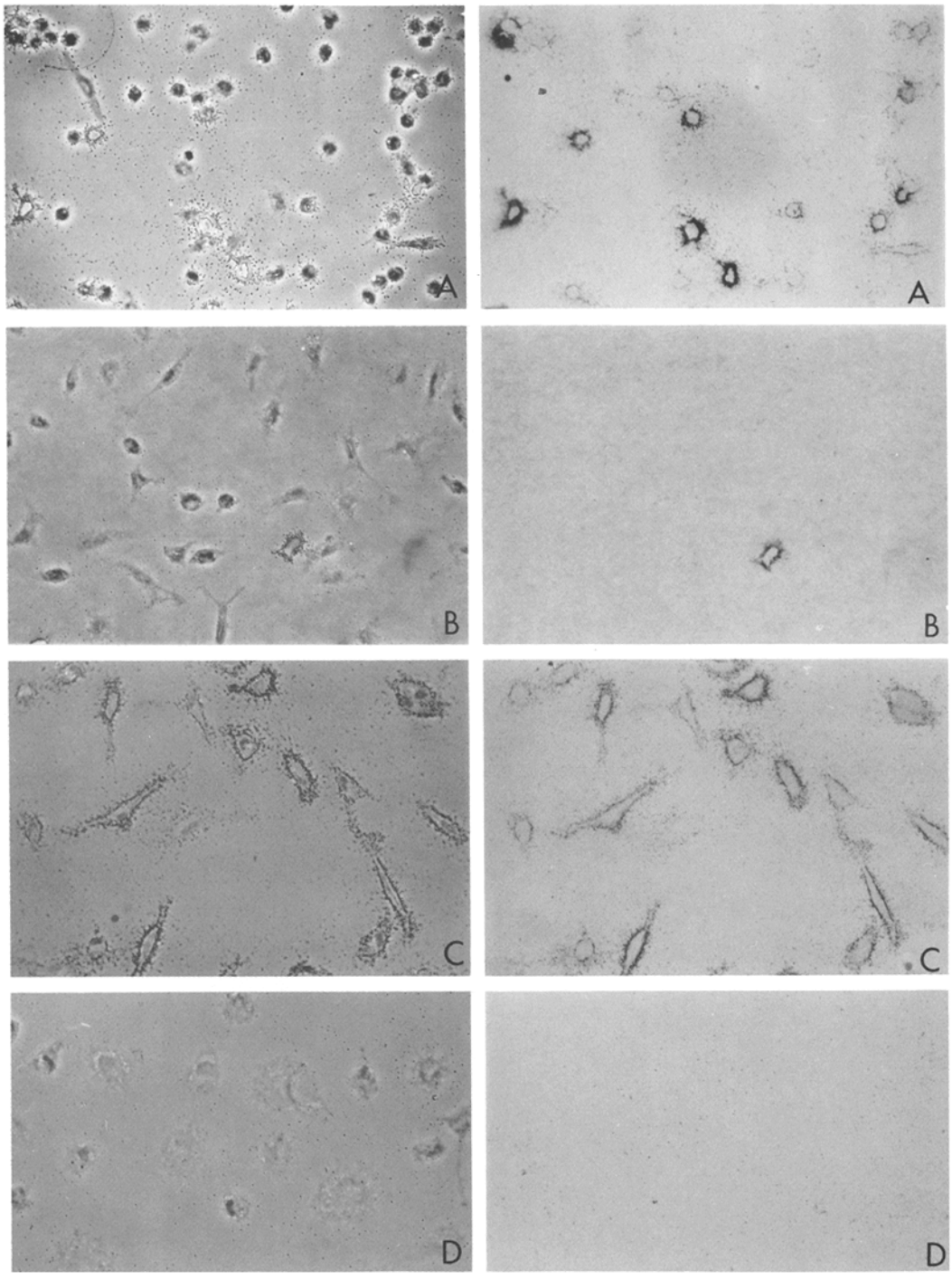

FIG. 2. Visualization of ^{125}I-B21-2 binding sites (Ia antigens) on cultured peritoneal Mϕ. Both phase-contrast (left) and bright-field (right) micrographs of the radioautograms are presented to visualize all cell profiles and those labeled with ^{125}I-anti-Ia. All micrographs are × 310. (A) Initial population of adherent cells in which a subpopulation of cells is Ia positive. (B) 1 d of culture in immune LK. Much of the Ia has disappeared. (C) 3 d of culture in immune LK. Ia has been re-expressed on most cells. (D) Control for nonspecific binding of ^{125}I-anti-Ia. CBA (I-A^k) Mϕ cultured in immune LK, did not bind clone B21-2 (anti-I-$A^{b,d}$). (E) 3 d of culture in D10 medium. Ia is virtually absent. (F) Companion culture to (E) in which immune LK was added from 68 to 84 h resulting in a rapid re-expression of Ia on most cells. (G) 3 d of culture in control LK. Ia has been re-expressed on a subpopulation of cells.

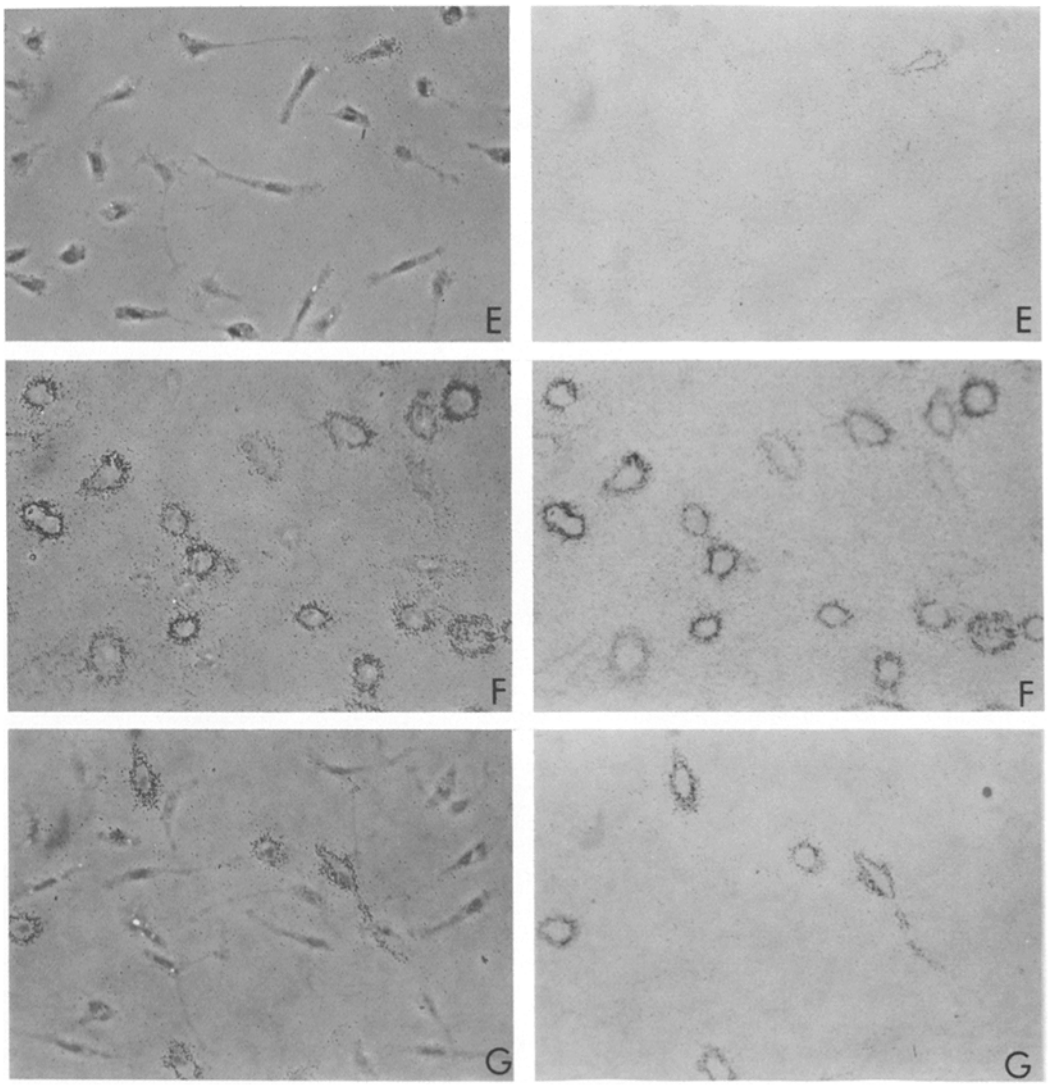

FIG 2E–G

If Mϕ were cultured for 3 d in D10, so that >95% of the cells were Ia negative (Fig. 2E), addition of LK again resulted in a re-expression of Ia on most cells (Fig. 2F). Enhanced binding was detected at 20 h, but not at 6 h (Fig. 1, Δ---Δ). In another set of cultures, LK was added for 3 d and then removed by washing. Ia persisted on LK-stimulated cells for at least 2 d and then fell (Fig. 1 ●---●), in contrast to the short-lived nature of Ia on freshly explanted Mϕ. We conclude that immune LK can induce most peritoneal Mϕ to express Ia in a stable form.

Media from HKT-immune boosted spleen cells (immune LK) were compared to control media from nonimmune spleen cells. Control LK at doses of 5, 10 (Table II), and 25% vol:vol (Fig. 3A) induced increases in Ia that were 20–50% that induced by immune LK. Somewhat larger increases were seen with media obtained from immune spleen not boosted with HKT (Table II), but it is likely that 2- to 3-wk *T. cruzi*-

TABLE II

Effect of Immune and Control LK On The Expression of Mϕ Plasma Membrane Antigens

Duration of culture	Source of LK (mouse spleen culture medium)	Nanograms monoclonal antibody bound					Micrograms protein/culture
		B21-2	B25-1	1.21J	2.6	24G2	
Experiment A							
h							
3	None	2.9	.90	6.8	.81	2.2	4.9
72	*T. cruzi* immune + HKT	9.0	2.5	6.9	1.3	3.3	9.1
72	*T. cruzi* immune + no HKT	5.3	1.9	5.2	1.2	3.6	7.8
72	*T. cruzi* nonimmune + HKT	3.0	1.4	7.1	1.8	3.5	7.1
72	*T. cruzi* nonimmune + no HKT	2.8	1.2	6.3	1.2	2.7	9.1
72	None	.09	.76	6.0	1.8	2.0	4.5
Experiment B							
h							
3	None	3.0	.75	7.4	.61	1.8	3.0
72	*T. cruzi* immune + HKT	4.9	.87	5.3	2.0	2.7	4.4
72	*T. cruzi* immune + no HKT	3.1	.82	5.4	1.5	2.6	4.4
72	*T. cruzi* nonimmune + HKT	1.6	.76	5.6	2.8	2.7	4.0
72	*T. cruzi* nonimmune + no HKT	2.0	.80	6.8	3.6	2.8	4.6
72	None	.25	.60	4.8	2.5	2.5	4.0

Two different sets (Experiments A and B) of LK were obtained from 48-h cultures of *T. cruzi*-immune or nonimmune spleen, maintained in the presence or absence of specific antigen HKT. LK was then used to supplement the D10 medium of peritoneal Mϕ cultures. In Experiment A, 10% vol:vol medium, and in Experiment B, 5% vol:vol was used. One third more cells were cultured in Experiment A. Binding studies were performed with five radioiodinated monoclonal antibodies using concentrations close to the level required to saturate all binding sites. An Fab fragment of clone 24G2 was used, whereas the other antibodies were DEAE-purified ascites from hybridoma-bearing mice.

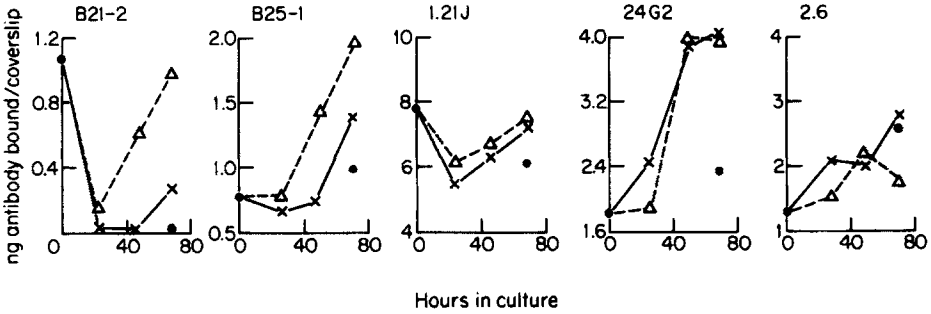

FIG. 3. Expression of five plasma membrane antigens on cultured peritoneal Mϕ at varying times in culture. Cultures were maintained in D10 medium (●), or D10 supplemented with immune (Δ---Δ) or control (×—×) LK. At varying time points, binding studies were performed with ^{125}I-monoclonal antibodies. Results are expressed as nanograms bound/cover slip of 1 × 10^5 cells. The five monoclonals, described in Materials and Methods, were B21-2 (anti-I-$A^{b,d}$); B25-1 (anti-H-$2D^d$); 1.21 J (Mac-1); 24G2 (anti-Fc); and 2.6 (another anti-Mϕ).

infected spleens harbor parasites. Kinetics studies in control LK showed that Ia first decreased and then was re-expressed usually at day 3 (Fig. 3A). By radioautography, it was determined that only a subpopulation of cells (20–40%) became Ia positive at all doses (Fig. 2G). If addition of control LK was delayed for 3 d, the Mϕ still re-expressed Ia at a level of 30% that induced by immune LK (not shown). Treatment

of immune or nonimmune spleen cells with anti-Thy-1 and complement before the culture period when LK was collected reduced LK activity by 60–90%. Finally, 2 samples of medium from concanavalin A-stimulated, nonimmune spleen cells were similar in activity to antigen-induced LK, causing a re-expression of Ia on most if not all cultured peritoneal Mϕ (data not shown).

Expression of Other Plasma Membrane Antigens In vitro. To determine if the effects of immune and control LK were restricted to Ia, we performed quantitative binding studies (Fig. 3; Table II) with four other monoclonal antibodies (Materials and Methods). Radioautographic data are not presented, because these four monoclonal reagents detected antigens that were expressed to a similar extent on all cultured Mϕ.

In contrast to Ia, expression of H-2D MHC determinants remained constant with culture at a level of 2×10^4–3×10^4 binding sites/cell. By day 2–3, both immune and control LK enhanced the level of H-2D, but immune LK usually induced larger and more rapid rises. The levels of 1.21 J antigen diminished 10–50% during culture in D10, but remained close to the starting level of 2.5×10^5–3.0×10^5 binding sites/cell in both immune or control LK. The trypsin-resistant Fc receptor (clone 24G2; 2×10^5 binding sites/cell) remained constant with culture in D10 and increased up to twofold with immune or control LK. These increases closely paralleled total cell protein/culture. Finally 2.6 antigen (3×10^4–4×10^4 binding sites/cell) increased two- to threefold during culture in D10 or in D10 with LK. In most experiments, the level of 2.6 was lowest in the presence of immune LK. We conclude that there is a wide range of behavior in the levels of plasma membrane antigens monitored simultaneously. The only moieties in which immune LK exerted selective enhancing effects were the MHC determinants—Ia and, to a lesser extent, H-2D.

Biosynthesis of Plasma Membrane Antigens. To determine if biosynthesis of Ia was induced by LK, we performed immunoprecipitation studies on cells radiolabeled with [^{35}S]methionine, usually on the third day of culture. Synthesis of Ia was readily detected in cells treated with immune LK (Fig. 4, lane 2), whereas synthesis of Ia was diminished or absent in cells not exposed to LK (Fig. 4, lane 4) or exposed to control LK (Fig. 4, lane 6). Synthesis of I-E-region alloantigens was also detected in LK-treated cells after immunoprecipitation with a (B10 × HTI)F_1 anti-B10.A(5R) anti-Ia 7+ alloantiserum (data not shown).

To establish that radiolabeling of Ia antigens was selectively increased in LK-treated cells, we extended the immunoprecipitation analysis with three other monoclonal antibodies. The non-MHC-linked polypeptides (1.21 J, 180,000 and 95,000 mol wt and 2.6, 21,000 mol wt) were radiolabeled similarly in the presence or absence of LK in four consecutive experiments. The class 1 MHC polypeptides (clone B25-1, 45,000 and 13,000 mol wt) showed a relative increase in radiolabeling in cells exposed to immune LK (Fig. 4; compare lanes 1 with 3 and 5). Because the radiolabeling of Ia (clone B21-2) and H-2D (clone B25-1) was increased relative to non-MHC plasma membrane antigens (clones 1.21 J and 2.6), we conclude that biosynthesis of MHC products is enhanced in LK-treated cells.

Stimulating Capacity of Ia-bearing Mϕ in the 1° MLR (Table III). 0.2×10^5–2×10^5 LK-induced Ia-bearing Mϕ were inactive as stimulators of the 1° MLR. Because the responder cells were harvested and pulsed with [^{3}H]thymidine in fresh medium, we verified that proliferating cells were not left behind on the Mϕ monolayers at the time of the proliferative assays. In contrast to Ia-bearing Mϕ, 0.3×10^5–1×10^5 DC

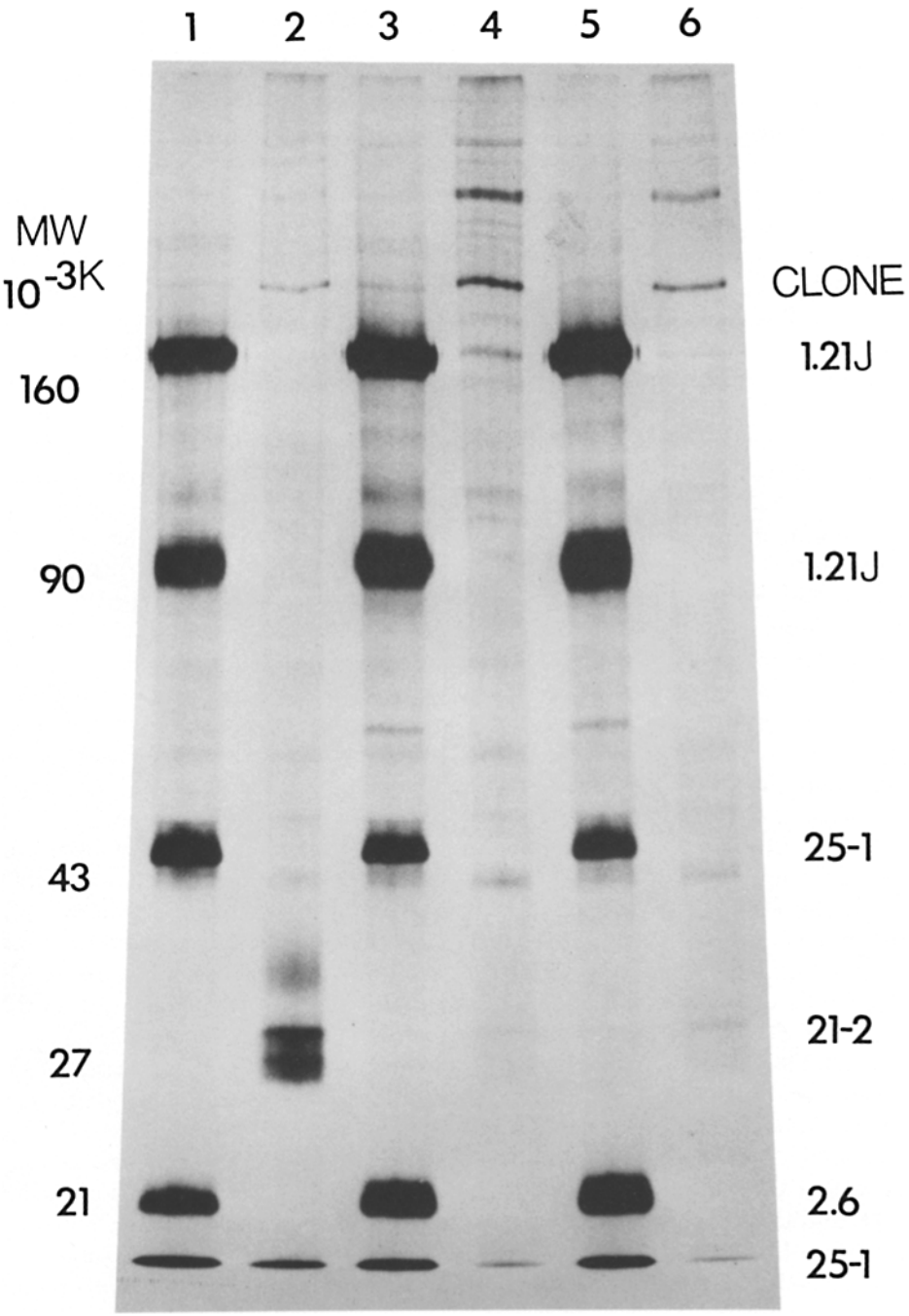

FIG. 4. Induction of Ia biosynthesis in Mϕ-treated with immune LK. Peritoneal Mϕ monolayers were labeled with [^{35}S]methionine at 50–64 h of culture in 10% vol:vol immune LK (lanes 1 and 2), D10 only (lanes 3 and 4), and 10% vol:vol control LK (lanes 5 and 6). Lysates were subject to immunoprecipitation with a cocktail of 1.21 J, B25-1, and 2.6 (lanes 1, 3, and 5) or with clone B21-2 anti-Ia (lanes 2, 4, and 6). The labeled antigens were separated on 4–11% gradient gels and visualized by radioautography. The polypeptides recognized by each monoclonal antibody have been described (8, 10) and are indicated on the right side of the gel, whereas the positions of molecular weight standards are on the left. Under conditions in which all cultures actively synthesize 1.21 J, B25-1, and 2.6 antigens (lanes 1, 3, and 5), immune LK dramatically increases the extent of Ia labeling (lane 2 vs. lanes 4 and 6). Synthesis of Ia was weak or undetectable if Mϕ were labeled after only 1 d in immune LK.

TABLE III

Lymphokine-induced Ia-bearing Mϕ Do Not Stimulate a 1° MLR

Stimulating cells	Proliferative response	
	Mouse serum	Fetal calf serum
	cpm [^{3}H]thymidine	
Experiment A		
1.5×10^5 Mϕ	535	3,295
1.5×10^5 + indomethacin	1,210	2,485
$1.5 \times 10^5 + 1 \times 10^4$ DC	1,545	4,785
$1.5 \times 10^5 + 1 \times 10^4$ + indomethacin	10,200	12,130
1×10^5 DC	21,165	38,890
3×10^4 DC	11,255	15,620
1×10^4 DC	7,690	9,210
1×10^4 + indomethacin	8,385	11,000
None	1,960	5,310
Experiment B		
2×10^5 Mϕ	3,705	1,170
2×10^5 + indomethacin	1,830	1,910
$2 \times 10^5 + 3 \times 10^4$ DC	15,220	16,700
$2 \times 10^5 + 3 \times 10^4$ + indomethacin	22,380	24,660
1×10^5 DC	34,870	30,040
3×10^4 DC	20,455	27,655
1×10^4 DC	10,700	10,665
1×10^4 + indomethacin	11,980	10,360
None	1,875	1,550

Ia antigens were induced on B6D2F$_1$ peritoneal Mϕ after 3 d of culture in immune LK (10% vol:vol). These Mϕ were then compared to Ia-bearing DC as 1° MLR stimulators for 4.5×10^6 nylon-wool-nonadherent DBA/2 spleen responders. Mϕ had 1.5×10^5 and 2×10^5 binding sites/cell and DC had 4×10^5 and 6×10^5 binding sites/cell in experiments A and B, as determined by Scatchard analysis. Proliferative responses were evaluated in cultures supplemented with 1% mouse serum or 1% fetal calf serum. In these experiments [^{3}H]thymidine uptakes were performed at day 3 with a 2-h pulse of radiolabel, 2 μCi/ml, 6.0 Ci/mM, and are expressed as counts per minute/culture of 4.5×10^6 cells. Similar findings were obtained in two experiments monitored on day 4 of the MLR.

induced maximal responses by day 3–4. DC expressed two to four times more Ia/cell as the LK-induced Mϕ (data not shown). It is difficult to ascribe differences in stimulating capacity to differences in the total level of Ia because 1×10^4 DC induced a large MLR and 2×10^5 Mϕ did not.

Addition of indomethacin to block the possible release of inhibitory prostaglandins did not increase Mϕ stimulatory capacity. Mϕ could inhibit the DC-stimulated MLR, however (Table III, experiment A). This inhibition was reversed by addition of indomethacin. Similar phenomena were observed previously in studies of the inhibitory effects of Mϕ on DC-mediated accessory function (3). We conclude that expression of Ia on Mϕ is not in itself sufficient to sensitize alloreactive T cells at the stimulator cell doses we employed.

Discussion

This study represents a fusion of two kinds of investigation. In one, we study Ia antigens on subpopulations of mouse cells (Mϕ, B cells, and DC) to try to relate expression of Ia to immunologic functions such as accessory cell activity and stimulation of the 1° MLR (3, 11). In the other, we investigate mechanisms and specific changes consequent to the activation of Mϕ by immune cells and their products (4–6). We report that LK from antigen-stimulated *T. cruzi*-immune spleen cells enhances the expression and synthesis of Mϕ Ia in a dose- and time-dependent fashion. Increase in Ia joins a long list of Mϕ properties induced by this source of LK including: spreading, secretion of plasminogen activator, capacity to release hydrogen peroxide, and ability to kill *T. cruzi* and tumor cells (4–6). These changes probably occur on most cells in the peritoneal Mϕ population. Enhanced levels of Ia, like secretion of plasminogen activator (4), is particularly sensitive to the addition of LK. As a consequence, it is not surprising that control LK from nonimmune spleen can exert similar but quantitatively less impressive changes. Nonimmune spleen may have cells sensitized to environmental antigens and may release the same mediators as immune-boosted cells. Alternatively, distinct factors may be present in control LK. We noted that the latter induced the expression of Ia only a subpopulation of cells, at all doses, in contrast to immune LK, which enhanced Ia on most, if not all cells (compare Fig. 2 C, F, and G).

In effect, expression of Ia on cultured peritoneal cells represents a surface marker that correlates with LK-induced Mϕ activation (4–6). The level of Ia may be a simple, quantitative, and rapid assay (Fig. 1 △---△; Fig. 2 E and F) for monitoring experiments characterizing the cells and mediators involved in the activation process. Mϕ obtained from the peritoneal cavities of immune-boosted mice also exhibit most parameters of Mϕ activation (13) and are strongly Ia positive (3, 14). However *in situ*, these changes may be mediated by LK and other factors that act both in the tissues (peritoneal cavity) or during Mϕ development (marrow and peripheral blood).

The availability of monoclonal antibodies was critical for the execution of these experiments. Binding data with a battery of monoclonal reagents indicated that expression of Ia, and to a lesser extent H-2D, was selectively enhanced by immune LK. The expression of three other plasma membrane antigens, detected by clones 1.21 J, 2.6, and 24G2, was not appreciably altered by immune relative to control LK (Fig. 3 and Table II). Similar quantitative data would be difficult to obtain from small numbers of adherent cells using cytotoxicity, indirect immunofluorescence, or absorption assays. In biosynthesis studies (Fig. 4), radiolabeling of Ia was selectively increased by immune LK, whereas radiolabeling of non-MHC polypeptides recognized by clones 1.21 J and 2.6 was similar in all cultures. Using the latter polypeptides as controls, we concluded that radiolabeling of Ia reflects an induction of Ia biosynthesis. Finally, the rat anti-mouse monoclonal antibody clone B21-2 is specific for the $Ia^{b,d}$ haplotype (Table I). Binding above the no-cell background was only seen on $Ia^{b,d}$ cells treated with LK [e.g., C57BL/6, DBA/2, $C6D2F_1$, B10A(5R)] and not Ia^{k} Mϕ (CBA; Fig. 2 C and D). By using LK from *T. cruzi*-immune CBA spleen cells, we ensured that the increase in Ia we observed was derived from the treated Mϕ and not adsorbed from the applied LK.

It is not clear if all members of the mononuclear phagocyte lineage increase Ia in response to LK. Our studies utilized proteose-peptone-elicited cells, because this

population reproducibly (15 consecutive experiments) increased Ia in the manner reported here. Less dramatic increases in Ia were usually found in LK-treated, resident peritoneal, and spleen Mϕ. Tissue culture itself also seemed to enhance susceptibility to LK. It took 24–48 h for LK to induce large increases in Ia on freshly explanted Mϕ, whereas cells that had been cultured 2–3 d exhibited one-half-maximal responses by 20 h (Fig. 1). Possibly, fetal calf serum and/or endotoxin contribute to the enhanced responsiveness of cultured Mϕ (15).

Several factors may contribute to enhanced levels of Ia. A primary mechanism seems to be the induction of biosynthesis in Mϕ exposed to LK. (Fig. 4). Other alterations that might occur in the presence of LK include: decreased degradation rates of pre-existing Ia; mobilization of Ia from pre-formed intracellular pools; and increased accessibility of surface Ia to the ^{125}I-anti-Ia probe.

Ia on freshly explanted peritoneal Mϕ exhibits several differences from Ia induced by immune LK. Endogenous Ia is heterogeneous in its distribution and short-lived in vitro, whereas LK-induced Ia is found on most cells and is more long-lived. The rapid and selective loss of Ia from the surface of peritoneal Mϕ is a puzzling finding. Other plasma membrane constituents turnover much more slowly with a $t_{1/2}$ of >24 h (16). Ia on explanted peritoneal cells may be analogous to myeloperoxidase; that is, it is actively synthesized by immature mononuclear phagocytes, persists for a finite period after cessation of synthesis, and then diminishes rapidly.

Previous studies on cultured rodent Mϕ have emphasized that Ia is a stable phenotype for a Mϕ subpopulation. However, most Mϕ Ia disappears rapidly from the cell surface during culture. LK then induces Ia on virtually all cells. These findings indicate that Ia on tissues Mϕ is not a constitutive trait. Like many other Mϕ properties, Ia may be altered by environmental factors, including tissue culture and immunologic stimuli.

How then would increased expression of Mϕ Ia contribute to the cellular immune response and to the biologic effects of LK? One hypothesis is that Ia is able to form some sort of complex with antigen (antigen processing or presentation) that is required for T cell stimulation. We did not test this possibility directly, but we did examine the ability of Ia-bearing Mϕ to stimulate a proliferative response in allogeneic T cells. The Mϕ were inactive, even when indomethacin was added to diminish the synthesis of inhibitory prostaglandins (Table III). Conceivably, LK-induced Mϕ inhibit their own functional capacities in vitro by an indomethacin-insensitive process. This is unlikely, because DC were fully capable of triggering the MLR in the presence of these Mϕ. Our data thus extend previous observations on the different functional properties of Ia-bearing DC and Ia-positive Mϕ generated *in situ* (3, 10). Ia on the surface of Mϕ may be intrinsically different from Ia on the surface of DC, or additional non-MHC factors are utilized by DC in functional assays.

We favor the idea that increased expression of Ia, and possibly H-2K/D, enhances the ability of T cells to interact directly with Mϕ. The T cells that secrete LK and that activate Mϕ are likely to be selected to recognize self-MHC determinants. Direct T-Mϕ interaction would enable the T cells and/or LK products to more efficiently activate Mϕ function. The initial generation of MHC-restricted effector cells however may be mediated by Ia-bearing DC (3). A similar requirement for MHC products mediating T-B interaction during B cell activation has been proposed (17).

Summary

Soluble products from antigen stimulated *Trypanosoma cruzi*-immune spleen cells enhanced the expression of Ia antigens on proteose-peptone-elicited mouse peritoneal macrophages (Mϕ). Acquisition of Ia paralleled Mϕ activtion, previously shown to be mediated by this same source of lymphokine (LK).

Expression of Ia and four other plasma membrane antigens was monitored by quantitative binding and radioautographic studies with ^{125}I-monoclonal antibodies. Immune LK selectively enhanced expression of Ia and, to a lesser extent, H-2D relative to control LK from antigen-stimulated noninfected spleen. The levels of three other non-major histocompatibility complex (MHC) antignes, including the trypsin-resistant Fc receptor, were similar in cells exposed to both sources of LK. As little as 1% immune LK induced one-half maximal expression of Ia. Kinetic studies revealed that much of the Ia on freshly explanted peritoneal Mϕ was lost during the 1st d of culture. In the continued presence of immune LK, Ia was re-expressed on virtually all Mϕ by the 2nd and 3rd d. Alternatively, >95% Ia negative populations were obtained by culturing the cells 3 d; then, addition of LK induced Ia on most cells within 1 d. Once induced, Ia persisted on the Mϕ surface for at least 2 d.

[^{35}S]methionine radiolabeling indicated that immune LK selectively increased radiolabeling of Mϕ Ia, again with other non-MHC-linked plasma membrane polypeptides as controls.

LK-induced Ia-bearing Mϕ were tested as primary mixed leukocyte reaction stimulators. 1×10^5–2×10^5 Mϕ did not stimulate 4.5×10^6 responding T cells, whereas 10^4 dendritic cells induced strong responses, as previously described. Because Ia-positive Mϕ do not actively sensitize T cells in a model immune response, we propose that Mϕ MHC products serve primarily as recognition sites for previously sensitized T cells, thereby enhancing T cell-mediated Mϕ activation.

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