MACROPHAGE HETEROGENEITY IN MAN

A Subpopulation of HLA-DR-bearing Macrophages Required for Antigen-induced T Cell Activation Also Contains Stimulators for Autologous-reactive T Cells

By HOWARD V. RAFF,* LOUIS J. PICKER, and JOHN D. STOBO‡

From the Section of Rheumatology, Department of Medicine, and the Howard Hughes Medical Institute, University of California, San Francisco, California 94143

Cells of the monocyte/macrophage line (collectively referred to as Mϕ)[1] fulfill a dual requirement for the initiation and expression of T cell reactivity (reviewed in references 1 and 2). First, Mϕ are required to present stimulating determinants in a manner suitable to initiate activation among reactive T cells. This presentation function requires appropriate display of conventional antigenic determinants in association with Mϕ glycoproteins (Ia in rodents, HLA-DR in man) coded for by immune response genes. Second, for full T cell activation to proceed, Mϕ are required to synthesize and to secrete soluble materials (collectively referred to here as lymphocyte-activating factor, which have recently been renamed interleukin[1] [LAF] [3–6]). Whereas the determinant presenting function of Mϕ is genetically constrained, the activity of LAF is not. The presence of Mϕ satisfying only one of these requirements is insufficient to support full T cell activation.

Although it is clear that Mϕ are required for maximal T cell reactivity, evidence exists that suggests that not all Mϕ are equally capable of mediating this requirement. For example, whereas effective presentation of conventional antigen to T cells requires that Mϕ display immune response gene products, not all Ia-bearing Mϕ in rodents or HLA-DR-bearing Mϕ in man can support antigen-induced activation of T cells (7–9). It has been reported that only those murine splenic Mϕ that display determinants coded for by two Ia subregions (I-A and I-E) can effectively present antigen or mitogen to T cells (7, 8). Moreover, limited cytolysis of human Mϕ with HLA-DR antibody yields a residual population of HLA-DR-bearing cells, which is not capable of supporting antigen-induced T cell proliferation or synthesis of lymphokines (9).

Previously we have reported that functional and biochemical heterogeneity exists among human peripheral blood Mϕ (10, 11). To define further this heterogeneity, we have developed a monoclonal antibody, that detects a 120,000-dalton determinant

* Research Associate of The Howard Hughes Medical Institute.

‡ Investigator of The Howard Hughes Medical Institute.

[1] *Abbreviations used in this paper*: ALA, anti-lymphocyte antibody; AMLR, autologous mixed lymphocyte reactivity; C′, complement; Con A, concanavalin A; FCS, fetal calf serum; ^{3}H-TdR, tritiated thymidine; LAF, lymphocyte-activating factors, which have recently been renamed interleukin 1; LIF, leukocyte inhibition factor; MIg, mouse immunoglobulin; MLC, mixed lymphocyte culture; MLR, mixed lymphocyte reactivity; Mϕ, monocyte/macrophage; PBMC, peripheral blood mononuclear cells; PPD, purified protein derivative; SDS-PAGE, polyacrylamide gel electrophoresis in sodium dodecyl sulfate; S.I., stimulation indices.

displayed, as determined by cytotoxicity and immunofluorescence, on 23–37% of the peripheral blood Mϕ from several HLA-DR-disparate individuals. This determinant is not detectable on T cells, B cells, or other nonlymphoid cells. In cytolytic assays, the Mac-120 antibody segregates Mϕ into two broad populations; those bearing both the 120,000 dalton determinant and HLA-DR, and those displaying only HLA-DR. The data to be presented suggest that only the former, and not the latter population, can function in the genetically restricted presentation of conventional antigen to T cells. Moreover, this Mac-120$^+$ population contains those Mϕ that are the most effective stimulators of an autologous mixed lymphocyte reaction (AMLR).

Materials and Methods

Cell Populations. Peripheral blood mononuclear cells (PBMC; 69 ± 2% T, 6 ± 2% immunoglobulin bearing, 22 ± 3% esterase positive) were obtained as previously described (12). Populations partially depleted of adherent cells and populations enriched for adherent Mϕ were obtained in the following manner. PBMC were suspended in RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) containing 10% nonheat-inactivated autologous serum and incubated on plastic Petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for 1 h at 37°C. Populations partially depleted of adherent cells (85% T, 6% B, 5–8% esterase positive) were removed by washing. Xylocaine (24 mM; Cutter Laboratories, Berkeley, Calif.) was then added to the dishes for 15 min at 23°C, and the adherent cells (90% viable, >95% esterase positive, >90% phagocytic) were removed by vigorous pipetting (11).

Production of Monoclonal Antibodies. Female BALB/c mice (Simonsen Labs Inc., Gilroy, Calif.) were first immunized intraperitoneally and subsequently intravenously with 8×10^6 adherent Mϕ. 3 d later, the spleens were removed and single cells suspensions fused to the murine plasmacytoma (NS-1) with polyethylene glycol (30%, VWR Scientific Inc., Subsid. of UNIVAR, San Francisco, Calif.), (13). The hybrids were suspended in DME-H21 (Cell Culture Facility, University of California, San Francisco, San Francisco, Calif.), supplemented with 10^{-2} M hypoxanthine, 5×10^{-5} M aminopterin, and 4×10^{-3} M thymidine (Sigma Chemical Co., St. Louis, Mo.) and 10% heat-inactivated horse serum (KC Biological, Inc., Lenexa, Kan.), and dispensed into flat-bottom microtiter plates. Fresh, supplemented media was added every 2 d. 2 wk later, culture fluid from each microtiter well was tested in an indirect radioimmunoassay with ^{125}I-F(ab)$'_2$ goat anti-mouse Ig, for binding to T-enriched, adherent cell-depleted vs. adherent cell-enriched populations (14). Hybrids producing antibodies that bound to both populations were discarded, whereas those containing Ig that bound only to the adherent Mϕ were saved for further investigation of their specificity.

Quantitation of cells reactive with monoclonal antibodies was performed using either direct immunofluorescence or indirect cytotoxicity (goat anti-mouse Ig plus low tox rabbit complement, [C′]; one:six dilution, Cedarlane Laboratories, Hornby, Ontario, Canada) in a ^{51}Cr release assay. To deplete cells reactive with the monoclonal antibody, a similar indirect cytotoxicity procedure was used. $5–10 \times 10^6$ target cells were incubated with 500 μg of affinity column-purified protein (Sepharose-goat anti-mouse Ig) at 4°C for 30 min. After washing, the cells were suspended in 500 μl of media containing 50, 25, or 6 μg of goat anti-mouse Ig and incubated for 30 min at 4°C. 100 μl of undiluted rabbit C′ was then added for 30 min at 37°C. Controls consisted of cells that were incubated in mouse IgG (MIg; Miles Laboratories, Inc., Kankakee, Ill.) in place of the monoclonal antibody. In no instance was either the MIg or the rabbit C′ found to alter cell function. The results presented represent those obtained with that concentration of goat anti-mouse Ig that resulted in the most complete removal of Mac-120-positive cells. In some experiments, PBMC or Mϕ were incubated with only antibody or MIg for 30 min at 4°C. The cells were then washed and are referred to as antibody-pulsed populations.

ALA refers to a monoclonal anti-lymphocyte antibody that depicts a determinant displayed by all lymphocytes and Mϕ.

Assays of Lymphocyte Reactivity. Assays to determine the proliferative reactivity of cells to mitogen (concanavalin A [Con A] two times recrystallized; New England Nuclear, Boston,

Mass.), antigens (*Candida albicans*, Hollister-Stier Labs, Livermore, Ontario, Canada; purified protein derivative PPD; Connaught Medical Research Laboratory, Willowdale, Ontario, Canada), alloantigens (HLA-D-disparate cells), and autologous stimulator cells were performed as previously described (12, 15). In each case, several concentrations of Con A and antigens or two concentrations of allogeneic or autologous stimulator cells were used with reactivity measured by the incorporation of tritiated thymidine (^{3}H-TdR; New England Nuclear) into DNA during the final 18 h of a 4-d (mitogen), 5-d (antigen), or 6- and 9-d (mixed lymphocyte-reactive [MLR]) culture. Results are expressed as either maximal Δ counts per minute (counts per minute in mitogen- or antigen-stimulated cultures minus counts per minute in nonstimulated cultures) or as stimulation indices (S.I.; counts per minute in cultures containing responder and stimulator cells/counts per minute in responder cells tested alone plus counts per minutes in stimulator cells tested alone).

Assay of leukocyte inhibitory factor (LIF) synthesis by PBMC was determined as described by Mauer et al. utilizing granulocytes obtained from a second, normal donor as indicator cells (16). Results from quadruplicate samples are presented as total random migration (cm^2) and as percent inhibition (random migration in the presence of supernate from antigen-stimulated PBMC/random migration in the presence of supernate from unstimulated PBMC).

Miscellaneous. HLA-DR typing was performed by the laboratory of Dr. Paul Terasaki using a panel of reagents, which depict seven HLA-DR specificities. Quantitation of HLA-DR-bearing cells was performed either by immunofluorescence or microcytotoxicity with sera from rabbits immunized with papain digests of the HLA-DR-bearing B cell line, GM-130. The sera were adsorbed with platelets from several HLA-A- and HLA-B-disparate donors and with the human T cell line CEM. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) analysis (nonreducing conditions) of biosynthetically labeled Mϕ membrane determinants reactive with the antisera demonstrated two bands of 23,000–25,000 and 30,000–35,000 daltons.

Results

Cell Populations Reactive and Mac-120. Utilizing an indirect radioimmunoassay, 400 hybridomas were screened for the production of antibody that bound only to Mϕ and not to T or B cells. Only one such hybridoma was found. Repeated (five times) cloning by limiting dilution yielded cells producing Ig of identical specificity, thus suggesting the presence of a single clone of antibody-producing cells. Antibody from this hybridoma was purified by affinity column chromatography (Sepharose plus goat anti-mouse Ig) and was used for all experiments. Photofluorographic analysis (7% SDS-PAGE, reducing conditions) of immune precipitates from solubilized, biosynthetically labeled adherent peripheral blood Mϕ demonstrated that the antibody was directed against a material with a molecular weight of 120,000 daltons. Thus, the antibody is termed Mac-120 (complete biochemical characterization of the 120,000-dalton determinant is the subject of another report [H. Raff and J. Stobo. Manuscript in preparation]). The presence of this 120,000-dalton determinant on different cell populations, as determined by immunofluorescence and cytotoxicity, is indicated in Table I. For comparison, immunofluorescent and cytotoxicity data obtained with a rabbit anti-HLA-DR serum are also presented. The Mϕ used in this study were obtained from individuals who were disparate at one or both HLA-DR loci. Note that (*a*) Mac-120 interacts with only Mϕ and not with other lymphoid or nonlymphoid cells, (*b*) only a portion (37 ± 2.8%) of Mϕ display easily detectable 120,000-dalton determinants as determined by the techniques used, and (*c*) virtually all peripheral blood Mϕ display HLA-DR indicating the presence of two broad subpopulations of Mϕ: Mac-120$^+$, HLA-DR$^+$, and Mac-120$^-$, HLA-DR$^+$ cells. Significant binding of Mac-120 to Fc receptors is unlikely in that other Fc receptor-bearing cells (B cells, neutrophils) were not depicted. Utilizing whole PBMC in the immunoflourescent

TABLE I

Populations of Cells Depicted by Mac-120 and Anti-HLA-DR

Population assayed	Mac-120		Anti-HLA-DR	
	Fl	Cytotox.	Fl	Cytotox.
Adherent Mϕ	37 ± 2.8	23 ± 8.8	94 ± 3	100
Phagocytic Mϕ	35 ± 2.9	ND	ND	ND
Normal T cells	0	1	0	3
Human T cell line (CEM)	0	0	0	0
Normal B cells	0	0	90	58
Human B cell line (HLA-DR-positive, GM 130)	0	3	93	92
Human histiocytic cell line	0	5	ND	ND
Normal neutrophils	2	ND	ND	ND
Human promyelocyte cell line (HL-60)	0	ND	ND	ND

The indicated cell populations were assayed for their reactivity with Mac-120 and anti-HLA-DR in either direct immunofluorescence (Fl) or indirect cytotoxicity (cytotox.). Phagocytic Mϕ refers to whole PBMC, which had been allowed to ingest latex particles and then quantitated for the frequency of fluorescence-positive, phagocytic cells. Normal T cells refers to E-rosette-positive, adherent cell-depleted populations (94% T cells, <1% esterase positive). Normal B cells refers to E-rosette-negative, adherent cell-depleted populations (95% Ig bearing, <2% esterase positive), which may also include null cells. Results represent the mean for 2 or mean ± SE for 6–12 determinations (ND, not done).

assay, Mac-120 detected 8.9 ± 1.1% of the mononuclear cells. Because whole PBMC contain 22 ± 3% Mϕ as determined by esterase staining, this would indicate that approximately 40% of Mϕ in untreated PBMC are Mac-120$^+$. This frequency is not substantially different from that obtained utilizing either adherent cells or PBMC that had been allowed to ingest latex (Table I). Therefore, it is unlikely that the procedures used to isolate or to depict Mϕ alter the observed frequency of Mac-120$^+$ Mϕ. The normal B cell population used in Table I contains E-rosette-negative, nonadherent cells and thus some null cells. Although Mac-120 failed to stain cells among this population, we did not directly assay populations enriched for null cells and thus cannot rule out the possibility that a portion of null cells are Mac-120$^+$. Mac-120 did not interact with two murine Mϕ lines (P388-D1 and WEHI-3) or with normal murine splenic Mϕ (data not presented).

Requirement for Mac-120$^+$ Mϕ in Mitogen- and Antigen-induced T cell Activation. The next series of experiments demonstrate that the subpopulation of Mϕ easily detectable by the Mac-120 reagent is required for mitogen- and antigen-induced activation of T cells. Mac-120 was tested for its ability to block in pulsing experiments, or to deplete cytolytically in indirect cytotoxicity, mitogen (Con A)- or antigen (*C. albicans*)-induced proliferation among PBMC that were first partially depleted of adherent Mϕ (5–8% esterase positive). Purified MIg was used as a control reagent. As demonstrated in Fig. 1 (top), Mac-120 substantially blocked, in all experiments except two, proliferative responses to both Con A and *C. albicans*. Cytolytic removal of Mac-120-bearing Mϕ consistently diminished both these T-dependent reactivities (Fig. 1 bottom). Although only maximal reactivities are presented, the responses noted at sub- or supraoptimal concentrations of mitogen and antigen were comparably reduced by either treatment

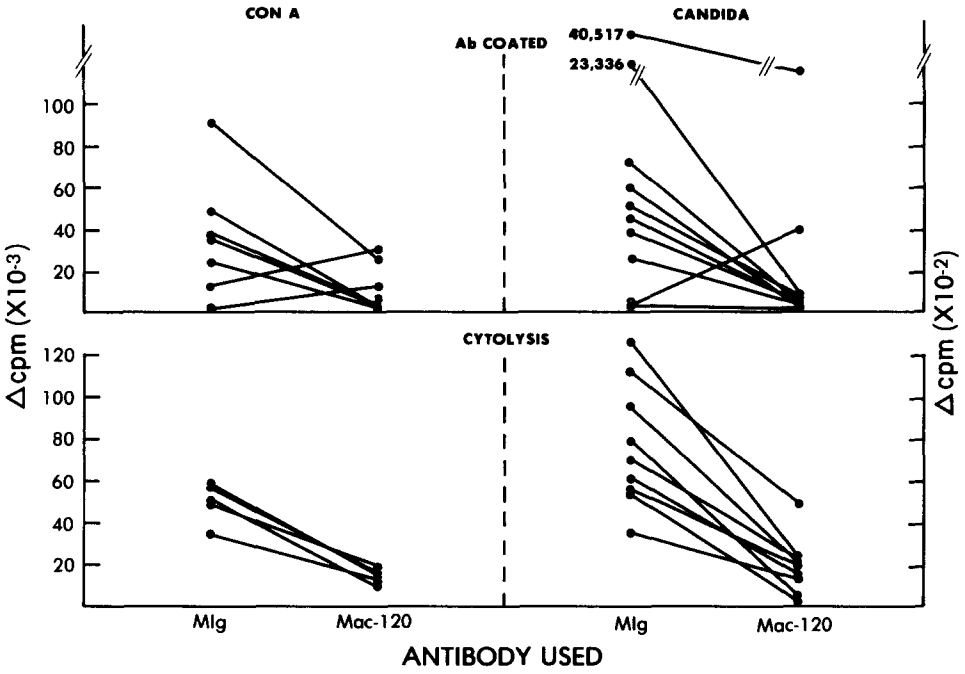

FIG. 1. Effect of Mac-120 on mitogen- and antigen-induced T cell proliferation. PBMC partially depleted of adherent cells were either incubated with MIg or Mac-120 for 30 min at 4°C and washed (Ab coated), or treated in an indirect cytotoxicity procedure with MIg or Mac-120 (cytolysis). 100,000 viable cells from each population were compared for the proliferative response to three concentrations of Con A or *C. albicans*. Results represent the maximal Δ counts per minute for 5–10 experiments.

with Mac-120. Removal of Mac-120-positive cells also reduced the response to another antigen, PPD (11,242 maximal Δcpm among MIg-treated vs. 927 maximal Δcpm among Mac-120-treated cells). Similar reduction in mitogen- and antigen-induced proliferation was noted when whole PBMC were cytolytically treated with Mac-120 (5,810 maximal Δcpm among MIg-treated PBMC vs. 656 maximal Δcpm among Mac-120-treated PBMC). However, PBMC that were first partially depleted of adherent Mϕ consistently yielded more complete removal of Mac-120$^+$ cells, a finding which may relate to the efficiency of the indirect cytotoxicity procedure. (Mac-120 detected in an immunofluorescent assay and lysed in a cytotoxicity assay 2–3% of the cells in the partially adherent cell depleted population. This represents 37–40% of the Mϕ present in this population.)

It was noted that the reactivity of populations only pulsed with Mac-120 could be restored to normal by incubating them at 37°C for 2 h before assay. In contrast, similar preincubation of cells cytolytically treated with Mac-120 did not result in restoration of antigen-induced proliferation (3,902 maximal Δcpm among MIg-treated and 196 maximal Δcpm among Mac-120-treated cells). This would indicate that the cytolysis experiments actually represent the result of removing Mac-120$^+$ cells and not simply antibody-mediated steric interference of their interactions with potentially reactive T cells.

To substantiate the requirement for Mac-120$^+$ Mϕ in antigen-induced T cell reactivity, another in vitro assay of T cell activation not requiring cell proliferation

was studied. Populations cytolytically treated with Mac-120 demonstrated a marked decrease in their ability to synthesize the lymphokine, LIF, in response to *C. albicans* (Table II).

Nature of the Requirement for Mac-120$^+$ Cells in T Cell Activation. The experiments outlined in Table III investigate the nature of the requirement for Mac-120$^+$ Mϕ in antigen-induced T cell activation. Whereas 10% untreated autologous Mϕ could reconstitute reactivity among Mac-120-depleted populations, 10% autologous Mϕ depleted of Mac-120$^+$ cells could not. This indicates that diminished antigen reactivity noted among Mac-120-treated populations represents a qualitative and not simply a quantitative deficiency of Mϕ. The relative inability of Mac-120-treated Mϕ to reconstitute T cell proliferation did not reflect suppression by toxic products liberated from dead cells. Mϕ treated with a monoclonal ALA so that 50% of the cells were lysed did reconstitute reactivity among Mac-120-treated responding cells (Table III, D). Mϕ from an individual disparate at both HLA-DR loci also failed to restore antigen reactivity, suggesting genetic restriction in the requirement for Mac-120$^+$ cells. Finally, antigen reactivity could not be reconstituted by soluble products obtained from autologous Mϕ cultured for 24 h in fetal calf serum (FCS). That these Mϕ culture fluids indeed contained T cell-activating factors (LAF) capable of substituting, in part, for Mϕ in antigen-induced T cell proliferation was supported by two observations. First, they reconstituted the antigen (ovalbumin)-induced proliferation among partially adherent cell-depleted, lymph node T cells from mice primed with ovalbumin (maximal ovalbumin response in the absence of culture fluids = 630 ± 30 vs. 19,160 ± 2,580 Δcpm in the presence of Mϕ culture fluids [L. Rosenwasser, H. Raff, and J. Stobo. Unpublished observations.]. Second, the culture fluids augmented the antigen-induced proliferation of the partially adherent cell-depleted populations used in Table III. The maximal response among partially adherent cell-depleted populations, which were treated with MIg and not reconstituted, was 3,902 vs. 7,726 Δcpm for the same population reconstituted with LAF-containing culture fluids. Allogeneic Mϕ also increased antigen reactivity among MIg-treated populations. This most likely represents the nongenetically restricted action of LAF synthe-

TABLE II

Requirement for Mac-120-positive Cells in the C. albicans-induced Production of Leukocyte Inhibition Factor

Reactive populations	Area of migration		Inhibition of migration	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
	cm^2		%	
MIg-treated PBMC plus media	45 ± 10	33 ± 12		
MIg-treated PBMC plus *C. albicans*	31 ± 5	6 ± 2	31	82
Mac-120-treated PBMC plus *C. albicans*	45 ± 9	37 ± 2	0	0

PBMC partially depleted of adherent cells were treated by indirect cytotoxicity with either MIg or Mac-120. Comparable concentrations of viable cells from each population were pulsed with either media only or *C. albicans* (one:six dilution) for 2 h and the amount of LIF liberated over a subsequent 48-h culture assayed utilizing fresh, normal leukocytes. Results are presented as the arithmetic mean ± SE area of migration or inhibition of migration for quadruplicate cultures in two experiments.

TABLE III

Reconstitution of Antigen-induced Proliferation among Mac-120-treated Populations

Responding population:		Reactivity to *C. Albicans*		
Antisera Rx	Reconstituted with	1/4	1/12	1/36
			(Δ *cpm*)	
A MIg	0	3,902	3,828	3,064
Mac-120	0	186	224	99
B MIg	Autologous Mϕ	6,785	4,198	3,352
Mac-120	Autologous Mϕ	3,363	3,032	1,435
C MIg	Mac-120 Rx autologous Mϕ	8,676	8,214	4,771
Mac-120	Mac-120 Rx autologous Mϕ	383	225	106
D MIg	ALA Rx autologous Mϕ	4,258	4,879	3,740
Mac-120	ALA Rx autologous Mϕ	4,164	4,577	3,552
E MIg	Allogeneic Mϕ	5,138	8,820	6,892
Mac-120	Allogeneic Mϕ	1,285	634	694
F MIg	LAF	7,726	6,389	4,680
Mac-120	LAF	152	124	87

PBMC partially depleted of adherent cells were treated by indirect cytotoxicity with either MIg or Mac-120; reconstituted with 10% of the indicated viable cells or LAF-containing culture fluids, and then tested for their proliferative reactivity to three concentrations of *C. albicans*. The results are presented as Δ counts per minute obtained in triplicate cultures from a single individual. LAF refers to a 50% concentration of culture fluids obtained from autologous Mϕ, which had been incubated at 37°C for 24 h in 10% FCS. ALA Rx autologous Mϕ refers to Mϕ cytolytically treated with suboptimal concentrations of a monoclonal anti-lymphocyte antibody so that 50% of the Mϕ was lysed. Allogeneic indicates an individual disparate at both HLA-D loci.

sized by the allogeneic Mϕ. The finding that allogeneic Mϕ increased, and did not suppress, antigen reactivity among MIg-treated cells argues against the possibility that a suppressive, allogeneic effect was responsible for their failure to reconstitute reactivity among Mac-120-treated populations.

A composite of experiments attempting to reconstitute antigen and mitogen reactivity among Mac-120-treated populations is presented in Fig. 2. Two points should be emphasized. First, the data obtained using cells from one individual (Table III) indicated that autologous Mϕ only partially (65%) reconstituted antigen induced proliferation. Complete reconstitution of antigen reactivity by autologous Mϕ was noted in the six experiments presented in Fig. 2. Second, in contrast with the situation noted for antigen reactivity, LAF partially reconstituted Con A-induced proliferation among Mac-120-treated populations.

Mac-120⁻ Mϕ Display HLA-DR. The studies to this point indicate that the Mac-120⁻ population of Mϕ cannot, by itself, support antigen-induced T cell activation. It is possible that they simply lack HLA-D determinants necessary for effective Mϕ-T cell interactions. The serologic data presented in Table I demonstrating that virtually all peripheral blood Mϕ display HLA-DR argue against this. To ensure that Mac-120⁺ Mϕ indeed displayed HLA-DR, Mϕ were cytolytically treated with MIg or Mac-120, reconstituted to comparable concentrations of viable cells, and compared,

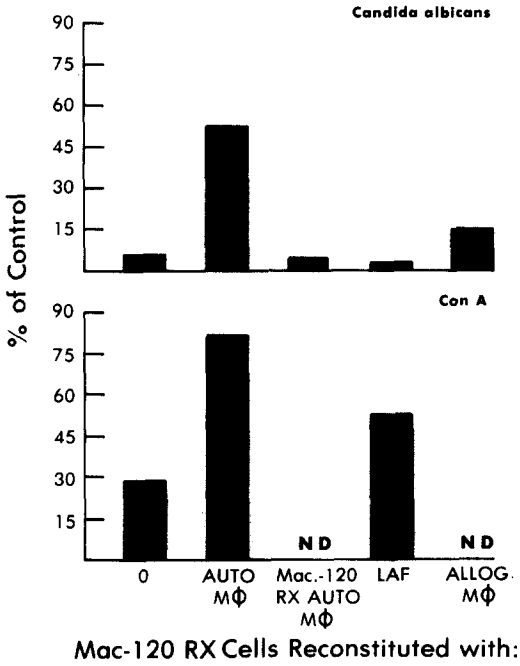

FIG. 2. Reconstitution of antigen- and mitogen-induced reactivity among populations depleted of Mac-120$^+$ cells. PBMC partially depleted of adherent cells were treated in an indirect cytotoxicity procedure with either MIg (control group) or Mac-120 (experimental group) and reconstituted with no cells (O), 10% autologous Mϕ, autologous Mϕ depleted of Mac-120$^+$ cells, culture fluids from autologous Mϕ, which contained LAF or 10% allogeneic (HLA-DR-disparate) Mϕ. The reactivity of these populations to three concentrations of either Con A or *C. albicans* were tested with results presented as a percentage of control reactivity ([maximal counts per minute in reconstituted Mac-120 Rx populations/maximal counts per minute in reconstituted MIg Rx cells] × 100). The results represent the mean ± SE for six experiments for two experiments.

at two ratios, for their ability to stimulate in an allogeneic MLR. In four experiments, which are representative of eight performed, Mϕ depleted of Mac-120$^+$ cells demonstrated no decrease in their ability to stimulate alloreactive T cells (Table IV; paired *t* testing of the S.I. for MIg Rx vs. Mac-120 Rx populations resulted in a *p* value of >0.3).

Thus a simple absence of HLA-DR determinants cannot explain the failure of Mac-120$^-$ Mϕ to support antigen-induced T cell activation. That the Mac-120$^+$ and Mac-120$^-$ populations may, in fact, differ in their expression of a surface determinant recognizable by T cells is suggested by the finding that these two populations differed in their ability to stimulate proliferative reactivity in an AMLR. This form of self-recognition is represented by the ability of appropriate concentrations of unmodified non-T cells to induce proliferation among autologous T cells during a 6- to 9-d culture (15). As indicated in Table IV, the same populations of Mac-120-treated cells that showed no decrease in their ability to stimulate alloreactive T cells, did demonstrate a statistically significant ($P < 0.01$) decrease in their ability to induce proliferation among autologous T cells. The differential ability of Mac-120-treated Mϕ to stimulate allo- vs. autoreactivity was not related either to nonspecific effects of dead cells or to the induction of suppressive influences capable of specifically inhibiting the AMLR. Mϕ treated with a monoclonal ALA so that 20% (experiment 1) and 80% (experiment

TABLE IV

Relative Ability of Mac-120-treated Mϕ to Stimulate an Allogeneic and Autologous MLR

Responder T	Stimulator Mϕ, antisera Rx		
	MIg	Mac-120	ALA
Experiment 1			
Allog.	19	21	20
Aut.	6.4	1.1	5.9
Experiment 2			
Allog.	43	42	43
Aut.	4.1	0.5	3.9
Experiment 3			
Allog.	57	48	ND
Aut.	12	1.6	ND
Experiment 4			
Allog.	26	27	ND
Aut.	15	3.1	ND

Mϕ were cytolytically treated with MIg, Mac-120, or a ALA. 50,000 and 100,000 viable cells from each treated population were compared for their ability to serve as stimulators in an MLC with 100,000 allogeneic (Allog.) or autologous (Aut.) T cells. Reactivity was measured by the incorporation of ^{3}H-TdR into DNA and is expressed as maximal stimulation index obtained in four experiments. The ALA was used in suboptimal concentrations so that 20% of the Mϕ were lysed in experiment 1 and 70% lysed in experiment 2. ND, not done.

2) of the cells were lysed showed no difference in their relative ability to serve as stimulators in an allogeneic or AMLR (Table IV). Mac-120-treated Mϕ did not inhibit the AMLR occurring among T cells and autologous B cells (S.I. for T plus B = 4.1; S.I. for T plus Mac-120-treated Mϕ = 1.6; S.I. for T plus B plus Mac-120-treated Mϕ = 3.7). The allogeneic and autologous MLR cultures were each harvested after 6 and 9 d of culture to ensure that the differences in stimulatory reactivities noted were not caused by differences in the kinetics of response. Whereas the data in Table IV are expressed as S.I., comparable relative differences were noted comparing the absolute counts per minute among cultures of Mϕ plus allogeneic T vs. Mϕ plus autologous T.

Discussion

The data presented in Table I demonstrate that by immunofluorescence and cytotoxicity the antibody Mac-120 depicts a determinant displayed only on approximately one-third of peripheral blood Mϕ. It should be emphasized that this does not necessarily indicate the existence of two distinct Mϕ populations that differ in regard to the absolute presence or absence of the 120,000-dalton determinant. The limits of sensitivity provided by the immunofluorescent and cytotoxicity assays may allow us only to detect Mϕ bearing a relatively high density of the Mac-120 determinant, whereas those bearing a reduced density could go undetected. This possibility is presently being explored utilizing more sensitive assays such as autoradiography and analysis with the fluorescence-activated cell sorter. Nonetheless, the functional studies utilizing conditions that resulted in maximal removal of Mac-120^{+} cells indicate the existence of functional heterogeneity among human peripheral blood Mϕ. The results

of these functional studies can be best interpreted within the framework provided by data obtained in rodents indicating a dual function, i.e., genetically restricted determinant presentation and synthesis of soluble activating substances for Mϕ in T cell activation (1, 2).

The data presented in Fig. 1 and Table II demonstrate that Mac-120 markedly diminishes antigen-induced T cell proliferation and lymphokine release. Utilizing a T cell-specific monoclonal antibody, we have demonstrated that both these reactivities are indeed T dependent (P. Hausman, H. Raff, R. Gilbert, L. Picker, and J. Stobo. Manuscript Submitted for publication.). This implies a difference between the ability of the Mac-120$^+$ and Mac-120$^-$ populations to support these reactivities. However, it is possible that both these populations are equally capable of supporting T cell activation but that interactions between the Mac-120 antibody and its ligand prevent effective collaboration between the Mac-120$^-$ population and reactive T cells. For example, immune complexes generated by the binding of Mac-120 to its 120,000-dalton ligand could attach to Fc receptors on Mac-120$^-$ cells and block their ability to provide signals necessary for T cell activation. Alternatively, the Mac-120 antibody could activate suppressive influences that inhibit T cell reactivity. Several observations argue against these possibilities. As indicated in the Results section, populations that were cytolytically treated with Mac-120 and then incubated for 2 h before assay to allow elution or internalization of immune complexes, did not demonstrate any increase in reactivity. In contrast, populations simply pulsed with Mac-120 and comparably incubated did. This suggests that in the cytotoxicity procedures, interactions between Mac-120, its ligand, and Fc receptors or blocking of crucial cell surface determinants did not account for the inability of Mac-120$^-$ cells to support T cell reactivity. Addition of Mϕ, which were either cytolytically treated (Table III, Experiment C) or simply pulsed with Mac-120 back to reactive populations, did not suppress antigen-induced proliferation (PBMC plus MIg-pulsed Mϕ = 6,963 vs. 8,425 Δcpm among PBMC plus Mac-120-pulsed Mϕ). Moreover, diminished antigen reactivity among Mac-120-depleted cells could be reconstituted by nontreated autologous Mϕ (Table III, Fig. 2). These studies indicate that Mac-120 did not activate nonspecific suppressive influences. When considered all together, the data support the conclusion that the Mac-120$^+$ population is required for antigen-induced T cell activation and that Mac-120$^-$ cells are intrinsically incapable of supporting this same reactivity.

Rosenwasser and Rosenthal (5) have demonstrated that partial removal of adherent Mϕ from immune rodent lymphoid cells markedly diminishes their T-dependent reactivity to multideterminant antigens. This deficiency could be reconstituted with soluble Mϕ products or intact allogeneic Mϕ (5). It has been postulated that the adherent cell-depleted immune cells contain sufficient autologous Mϕ to function in genetically restricted determinant presentation, but not in the production of soluble activating factors (e.g., LAF). By analogy, we reasoned that if Mac-120 removed a subpopulation of LAF-producing Mϕ, then the diminished T cell reactivity among populations containing Mac-120$^-$ cells should be restored by the addition of either LAF or allogeneic cells. As indicated in Table III, this was not the case. We have not directly tested the ability of Mac-120$^+$ Mϕ to effectively present conventional antigen to T cells. However, within the framework provided by what is known concerning the requirements for Mϕ in antigen-induced T cell activation, the reconstitution experiments indirectly support the conclusion that Mac-120$^+$ Mϕ can function as determinant-presenting cells whereas Mac-120$^-$ cells cannot. Although not presented, we

have evidence suggesting that Mac-120$^+$ cells can synthesize LAF. Enhanced synthesis of this material induced by the Mac-120 antibody could explain the occasional enhancement of mitogen- and antigen-induced proliferation noted among populations pulsed with Mac-120 (Fig. 1, top).

Differences existing among the Mac-120$^+$ and Mac-120$^-$ Mϕ, which are related to their relative ability to support antigen-induced T cell activation, are not clear. In rodents and man, it can be demonstrated that Mϕ remaining after cytolytic treatment with antisera directed against Ia or HLA-DR determinants, respectively, are deficient in their ability to support antigen-induced T cell proliferation (7, 9). Presumably, these residual Mϕ bear a density of Ia or HLA-DR, which is insufficient for them to function in antigen presentation. It could be argued that a similar situation exists among Mϕ remaining after cytolysis with Mac-120. That is to say, the density of the 120,000-dalton determinant displayed by Mϕ might parallel that of HLA-DR. Mϕ remaining after cytolysis with Mac-120 might not bear sufficient HLA-DR determinants for them effectively to present antigen to T cells. The demonstration (Table IV) that Mϕ remaining after cytolysis with Mac-120 do not demonstrate any decrease in their ability to act as stimulators in an allogeneic MLR indicates that they do not completely lack HLA-D gene products. Quantitative adsorbtion studies demonstrate that, when compared with whole Mϕ populations, Mac-120$^-$ cells bear a slightly increased and not a decreased density of HLA-DR determinants. Mϕ remaining after cytolysis with Mac-120 could adsorb more lytic activity from HLA-DR antisera than could intact peripheral blood Mϕ (H. Raff and J. Stobo. Unpublished observations.). Thus, the inability of Mac-120$^-$ cells to support antigen-induced T cell reactivity cannot be simply related to a quantitative decrease in HLA-DR expression.

That the Mac-120$^+$ and Mac-120$^-$ populations may differ with respect to their display of some determinant required for interactions with T cells is suggested by the data presented in Table IV. Only Mac-120$^+$ and not the Mac-120$^-$ population contains those Mϕ that are effective stimulators in an AMLR. The relationship of interactions between T cells and non-T cells participating in the AMLR to those occurring among immunocompetent cells as well as the nature of the stimulating signals that initiate the AMLR are not firmly established. Recently, we have demonstrated that the AMLR is heterogenous and involves the proliferation of at least two distinct responder T cells that recognize signals from distinct stimulator cells. Most importantly, T cells that proliferate in response to stimulation by autologous Mac-120$^+$ Mϕ are required for the reactivity to conventional antigen. In other words, that portion of the AMLR represented by interactions between T cells and Mac-120$^+$ Mϕ reflects interactions occurring between antigen-reactive T and antigen-presenting Mϕ. Our laboratory as well as others have presented evidence to indicate that proliferation of T cells in the AMLR requires recognition of HLA-D-linked gene products, although further studies are required to confirm this (15, 17). When considered in light of the demonstration that Mac-120$^-$ cells can stimulate in an allogeneic but not in an autologous mixed lymphocyte culture (MLC), this suggests that there may be qualitative differences in the expression of HLA-D gene products among Mac-120$^+$ cells. This could be represented by differences in the molecular species of HLA-D displayed or perhaps by differences in the spatial orientation and surface mobility of membrane HLA-D determinants. Alternatively, it is possible that the Mac-120$^+$ Mϕ bear some determinant other than HLA-D, which is required for appropriate interactions with T cells. The finding that the Mac-120 antibody can block, in pulsing experiments, antigen-induced T cell activation (Fig. 1) and the

ability of Mac-120$^+$ cells to stimulate in an autologous MLC, but does not affect their ability to stimulate in an allogeneic MLC (data not presented) is intriguing in this regard. We should point out that not enough is yet known concerning the relative morphology, adherence properties, phagocytic capabilities, and Fc receptor expression among Mac-120$^+$ vs. Mac-120$^-$ cells to determine if the former population resembles the dendritic cells described in murine systems by Steinman et al. (18). The murine dendritic cells and the human Mac-120$^+$ are similar with regard to some functional parameters.

Whereas the Mac-120$^+$ cells are also required for activation of T cells by mitogen (Con A), their requirement in this reactivity is somewhat different from that noted for activation of T cells by antigen. LAF partially reconstituted Con A reactivity among Mac-120-depleted cells. This is consistent with the findings of Rosenstreich et al. (19) indicating that only a portion of mitogen-reactive T cells actually require presentation of mitogen by Mϕ. Activation of other T cells can be induced by their direct interaction with mitogen provided that LAF is also present (19).

In summary, the finding that Mϕ required to effectively present conventional antigen to reactive T cells are included in the same population capable of stimulating in an autologous MLC but does not necessarily include all Mϕ capable of stimulating in an allogeneic MLC provides an interesting association useful for exploring the biology of functional interactions between Mϕ and T cells.

Summary

Utilizing somatic cell hybridization, we have developed a monoclonal antibody that interacts only with cells of the monocyte/macrophage (Mϕ) line and not with other myeloid or lymphoid cells. This antibody detects a 120,000-dalton determinant present on 37 ± 2.8% of the peripheral blood Mϕ from several (HLA-DR)-disparate individuals and only depicts a subpopulation (~30%) of HLA-DR-bearing Mϕ from any single subject. Cytolytic removal of this subpopulation of HLA-DR-bearing cells markedly diminishes antigen-induced T cell reactivity, a deficiency that can be reconstituted with autologous Mϕ but not with either their soluble products containing lymphocyte-activating factor or with intact HLA-DR-disparate Mϕ. Whereas Mϕ bearing both the 120,000-dalton determinant and HLA-DR serve as effective stimulators for autologous mixed lymphocyte reactions, Mϕ bearing only HLA-DR determinants do not. However, this latter population of Mϕ can stimulate proliferation among alloreactive T cells. These studies indicate that the Mac-120 monoclonal antibody detects a subpopulation of HLA-DR-bearing Mϕ that is required for the genetically restricted presentation of conventional antigen to reactive T cells. Within the Mϕ population, these Mac-120$^+$ cells constitute the most effective stimulators for autologous mixed lymphocyte reactions.

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