REGULATION OF MURINE MACROPHAGE Ia-ANTIGEN EXPRESSION BY PRODUCTS OF ACTIVATED SPLEEN CELLS

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Two subpopulations of macrophages have been identified on the basis of surface Ia-antigen expression. Ia-containing (Ia^+)[1] macrophages predominate in the spleen (1, 2), thymus (3), and liver (4), whereas Ia-deficient (Ia^-) macrophages (>90% Ia^-) are found in peritoneal exudates (1, 2, 5). T lymphocyte activation, as in the mixed leukocyte reaction MLR (6–9), antigen- or mitogen-induced proliferation (10–12), or the production of lymphokines (13) requires interaction with Ia^+ macrophages. Macrophages depleted of Ia^+ cells by anti-Ia and complement treatment are unable to act as accessory cells in the activation of T lymphocytes.

Limited evidence has suggested that Ia^+ and Ia^- macrophage subpopulations are interconvertible. Splenic macrophages cultured in vitro for several days were predominantly Ia^- (2). Conversely, Beller et al. (14) have reported that intraperitoneal injections of either *Listeria monocytogenes*, *Listeria*-immune T cells plus heat-killed organisms, hemocyanin, or macrophage-T cell-culture supernate resulted in an increase in the percentage of Ia^+ macrophages in the peritoneal exudate. These data, however, cannot distinguish between several possible explanations: i.e., (*a*) conversion of Ia^- to Ia^+ macrophages; (*b*) selective proliferation of Ia^+ peritoneal macrophages; or (*c*) recruitment of Ia^+ macrophages from other sites to the peritoneum. Additionally, Lee and Wong (15) have reported that bone marrow cells cultured in conditioned media that contained colony-stimulating factor (CSF) activity were partially Ia^+. However, because the percentage of Ia^+ macrophage precursor cells could not be determined (because of contaminating T and B cells), it is not certain whether the conditioned media induced Ia-antigen expression or stimulated the proliferation of both Ia^- and Ia^+ precursor cells.

Recently we reported that a considerable proportion of peritoneal exudate macrophages cultured in vitro with a concanavalin A (Con A)-stimulated spleen cell supernate (Con A sup) became Ia^+, whereas Con A-supplemented spleen cell supernate (Control sup)-treated macrophages remained Ia^- (16). This paper demonstrates that the appearance of Ia-antigen-bearing phagocytic macrophages in Con A sup cultures

* Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Department of Zoology, University of Maryland, College Park, Md.

[1] *Abbreviations used in this paper:* CI, cytotoxicity index; Con A, concanavalin A; Con A sup, Con A-stimulated spleen cell supernate; Control sup, Con A-supplemented (unstimulated) spleen cell supernate; CSF, colony-stimulating factor; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; Ia^+, Ia-antigen containing; Ia^-, Ia-antigen deficient; MGF, macrophage growth factor; MLR, mixed leukocyte reaction; PEC, peritoneal exudate cells; [^{3}H]TdR, [^{3}H]thymidine.

results from endogenous expression. Additionally, phenotypically converted Ia^+ peritoneal exudate macrophages also became more functional in the Ia-dependent MLR.

Materials and Methods

Animals. C3H/HeN (Ia^k) mice were obtained from the Division of Research Resources, National Institutes of Health (NIH), Bethesda, Md. BALB/c (Ia^d) and DBA/2 (Ia^d) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All mice were used at 8–12 wk of age. Mice were fed standard lab chow and water *ad libitum*.

Alloantisera. Two alloantisera were used in this study. A.TH anti-A.TL (Cedarlane Laboratories, Ltd., Accurate Chemical & Scientific Corp., Hicksville, N. Y.) detects both the Ia^k and Ia^d haplotypes. This antiserum has been demonstrated to be a reliable anti-Ia serum in several laboratories (1, 3, 10, 13). BALB/c anti-CKB serum (clone 11-5.2; specificity 2; B-D FACS Systems, Mountain View, Calif.) is a γ2B immunoglobulin reactive to the I-A subregion. This monoclonal antiserum is reactive to the $I\text{-}A^k$, but not the $I\text{-}A^d$ haplotype.

Preparation of Spleen Cell Supernates. Con A sup and Control sup were prepared by a modification (17) of the method of Nogueria and Cohn (18). Briefly, spleens were removed, and a cell suspension was prepared by passing the spleens through a sterile stainless steel wire mesh into RPMI-1640 medium (NIH Media Unit, Bethesda, Md.) supplemented with 8×10^{-4} M glutamine (NIH Media Unit), 15 mM Hepes buffer (NIH Media Unit), 5×10^{-5} M 2-mercaptoethanol (Eastman Kodak Co., Rochester, N. Y.), 2% fetal calf serum (FCS; Armour Pharmaceutical Co., Phoenix, Ariz.), and antibiotics. After washing, 10^8 spleen cells were incubated with (Con A sup) or without (Control sup) 3 μg/ml Con A (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) in 6.5 ml of media for 48 h at 37°C in a humidified 5% CO_2 atmosphere. 3 μg/ml Con A was added to control cultures after incubation, the cultures were harvested, and the spleen cells removed by centrifugation. The supernates were then absorbed with Sephadex G-50 for 1 h at 37°C to remove the Con A. The suspension was recentrifuged and the supernates filtered through 0.45-μm Millex filters (Millipore Corp., Bedford, Mass.). Supernates were aliquoted and stored at −20°C until use.

Macrophage Collection and Culture. Peritoneal exudate cells (PEC) were induced by intraperitoneal injection of 2 ml of 3% sterile thioglycollate broth (NIH Media Unit). 3–4 d later the cells were collected by peritoneal lavage with 5 ml of Hanks' balanced salt solution (HBSS; NIH Media Unit), washed, and resuspended in RPMI-1640 that contained 2% FCS and antibiotics. 1.25×10^4 PEC in 0.25 ml were plated/well in Lab-Tek tissue culture chamber slides (Miles Laboratories, Inc., Naperville, Ill.) and were cultured for at least 3 h at 37°C in a humidified 5% CO_2 atmosphere. The monolayers were washed three times in RPMI-1640 that contained 2% FCS, resuspended in 0.4 ml enriched McCoy's 5-A medium for granulocyte/macrophage culture (19) that contained dilutions of the Con A sup or Control sup, and cultured at 37°C in 5% CO_2 for 7 d.

Detection of Ia^+ Macrophages. Culture supernates were removed, replaced with 0.1 ml of RPMI-1640 that contained antiserum (1:20 dilution of A.TH anit-A.TL; 1:200 dilution of BALB/c anti-CKB), and incubated for 45 min at 4°C. Subsequently the antiserum was replaced with 0.1 ml of a 1:20 dilution of Low-Tox-M Rabbit Complement (Cedarlane Laboratories, Ltd.), and the cultures incubated at 37°C for 45 min. Cytotoxicity was determined by trypan blue exclusion (20), in which at least 100 cells/well in triplicate were examined under 100 × magnification, and expressed as a mean cytotoxicity index (CI):

$$CI = 100 \times \frac{\text{percent cytotoxicity (antiserum and complement)} - \text{percent cytotoxicity (complement alone)}}{100\% - \text{percent cytotoxicity (complement alone)}}$$

MLR. Graded numbers of C3H/HeN (Ia^k) PEC were collected and incubated with supernate in enriched McCoy's 5-A medium for 5 d as previously described, with the exception that TC-96 plates (Costar Data Packaging, Cambridge, Mass.) were used. Duplicate cultures were prepared, one of which was used to determine the percentage of Ia^+ PEC after incubation. The supernate-containing media in the remaining plate was replaced by 1.6×10^5 DBA/2 splenic responder cells in 0.2 ml of RPMI-1640 supplemented with 10% FCS, 5×10^{-5} M 2-

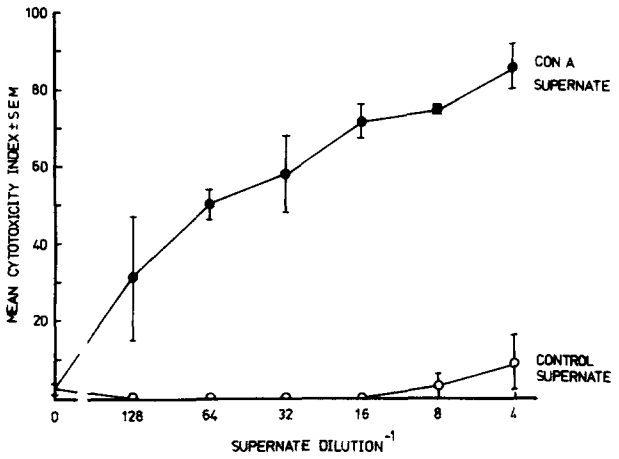

FIG. 1. Comparison of Con A sup and Control sup stimulation of Ia-antigen expression by PEC. Adherent C3H/HeN (Ia^k) PEC were incubated with dilutions of Con A sup or Control sup prepared from C3H/HeN spleen cells for 7 d, and the Ia-antigen expression determined from the cytotoxic effect of A.TH anti-A.TL serum and complement treatment, as assayed by trypan blue exclusion. Data are reported as the mean CI ± SEM of triplicate cultures in a representative experiment. The Ia-antigen expression of Con A sup- and Control sup-incubated cultures were significantly different ($P < 0.05$) at dilutions <1:128.

mercaptoethanol, and antibiotics. Cultures were incubated for 4 d at 37°C in a humidified 5% CO_2 atmosphere, and subsequently pulsed for 4 h with 1 μC of [^{3}H]thymidine/well ([^{3}H]TdR; 1.9 Ci/Mm sp act; Becton Dickinson, Rockville, Md.). Cultures were harvested onto glass fiber filters with a Mash II automatic harvester (Microbiological Associates, Walkersville, Md.), air dried, and the radioactivity counted by liquid scintillation. Values were expressed as a stimulation index, defined as the [^{3}H]TdR incorporation by mixtures of stimulator and responder cells divided by that of stimulator and responder cells cultured individually.

Macrophage Proliferation Assay. PEC were collected as described and 2×10^4 cells plated/well in TC-96 plates. The cells were cultured in 0.2 ml of enriched McCoy's 5-A medium with or without maximally active dilutions of Con A sup or Control sup. On days 1, 3, and 6 of culture, the PEC were pulsed for 6 h with 1 μCi [^{3}H]TdR/well, as described above, and the thymidine incorporation measured by liquid scintillation.

Macrophage Growth Factor Assay. Macrophage Growth Factor (MGF) was measured by a modification[2] of the method described by Stewart and Lin (21).

Statistics. Data were tested for significant differences by single classification analysis of variance.

Results

Incubation of PEC with Con A Sup Increases Ia-antigen Expression. C3H/HeN (Ia^k) PEC, which consisted of 98% macrophages as determined by nonspecific esterase staining and 97% phagocytic cells by latex bead ingestion, were cultured with Con A sup or Control sup prepared from C3H/HeN mice and the percentage of Ia^+ PEC determined using A.TH anti-A.TL serum (Fig. 1). A dose-dependent increase in the percentage of Ia^+ cells (up to 81%) resulted from incubation with Con A sup, whereas Control sup-incubated cells remained <10% Ia^+. Although Con A sup-induced Ia antigen was always dose-dependent, the maximum percentage of Ia^+ PEC obtained

[2] Moore, R. N., P. S. Steeg, D. N. Männel, and S. E. Mergenhagen. Role of lipopolysaccharide in regulating colony-stimulating factor dependent macrophage proliferation in vitro. Manuscript submitted for publication.

ranged from 12 to 33% using BALB/c anti-CKB serum, and supernates prepared from different murine strains varied in potency. For example, supernates prepared from BALB/c spleens typically stimulated Ia-antigen expression only 10–20% above controls, whereas DBA/2 and C3H/HeN supernates elicited greater responses (Figs. 1 and 3).

The kinetics of Con A sup-stimulated Ia-antigen expression are shown in Fig. 2. PEC were incubated with a maximally active dose of Con A sup or Control sup, and the percentage of Ia^+ cells determined daily using A.TH anti-A.TL serum. Ia-antigen expression increased substantially during the first 4 d of culture, and remained relatively constant up to 3–5 d thereafter. The 8–10% of PEC initially expressing Ia-antigen, when cultured in Control sup, consistently became Ia^- after several days in culture (Fig. 2).

Increases in the Percentage of Ia^+ PEC Are a Result of Endogenous Expression of Ia Antigen. The possibility that increased Ia-antigen expression might be a result of adsorption of Ia antigen present in the Con A sup was studied. This was investigated by incubating C3H/HeN (Ia^k) PEC with Con A sup and Control sup prepared with DBA/2 (Ia^d) spleen cells. Ia-antigen expression was then determined with A.TH anti-A.TL serum, which recognizes both the d and k haplotypes, and BALB/c anti-CKB serum, which recognizes the k but not the d haplotype (Fig. 3). Treatment of Con A sup-cultured PEC with antiserum and complement resulted in a supernate-dose-dependent cytotoxic response (maximum CI of 81% with A.TH anti-A.TL serum and 33% with BALB/c anti-CKB serum). The results using BALB/c anti-CKB serum and complement established that at least 33% of the Ia-antigen expression by PEC was endogenous. Macrophages cultured in Control sup did not show significant cytotoxicity when treated with either antisera.

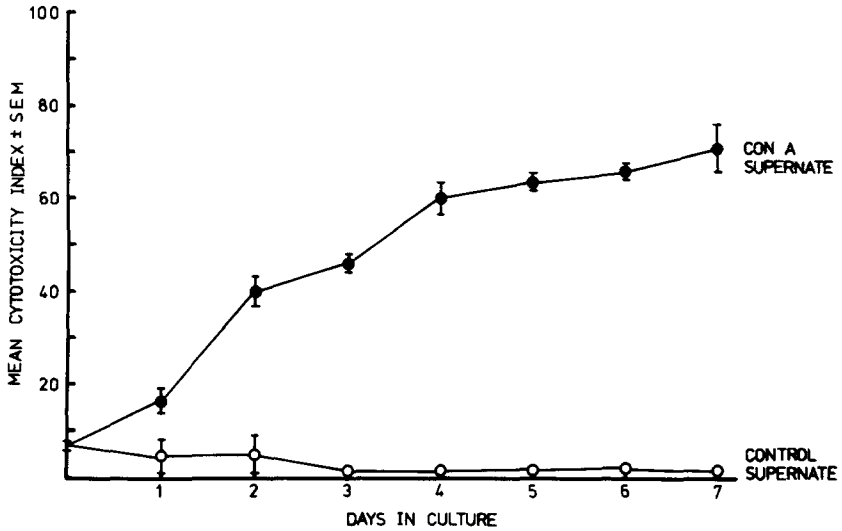

FIG. 2. Kinetics of Con A sup-stimulated Ia-antigen expression. C3H/HeN (Ia^k) adherent PEC were incubated with a 1:18 dilution of Con A sup or Control sup, prepared from C3H/HeN spleen cells. Ia-antigen expression was determined daily from the cytotoxicity of A.TH anti-A.TL serum and complement. The Ia-antigen expression of Con A sup- and Control sup-incubated cultures were significantly different ($P < 0.05$) after 1 d of culture.

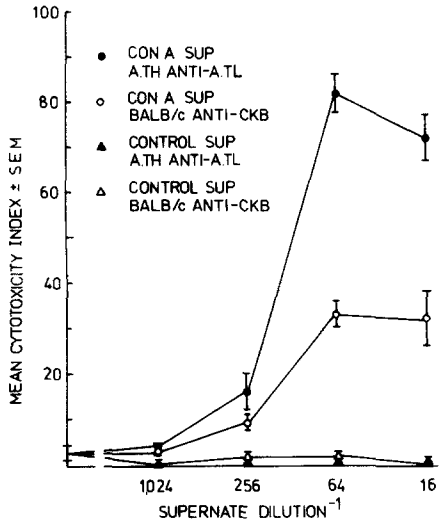

FIG. 3. Endogenous expression of Ia antigens by PEC. C3H/HeN (Ia^k) adherent PEC were incubated for 7 d with dilutions of Con A sup (circles) or Control sup (triangles) prepared from DBA/2 (Ia^d) spleen cells. Ia-antigen expression was determined with either A.TH anti-A.TL (closed) or BALB/c Anti-CKB (open) sera and complement in a representative experiment. Con A sup- and Control sup-induced Ia-antigen expression were significantly different ($P < 0.05$) at dilutions <1: 1,024.

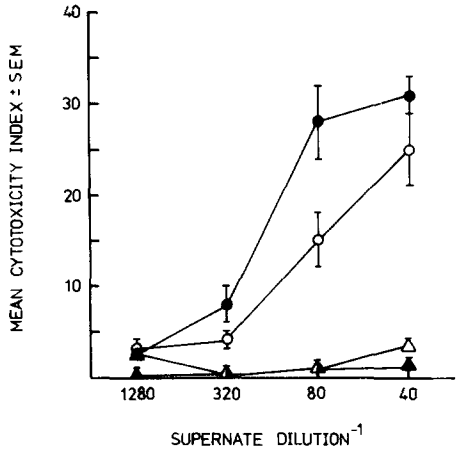

FIG. 4. Con A sup-stimulated conversion of Ia^- to Ia^+ PEC. C3H/HeN (Ia^k) adherent PEC were treated with A.TH anti-A.TL serum and complement (closed) or complement alone (open) and subsequently incubated for 7 d with dilutions of Con sup (circles) or Control sup (triangles) prepared from DBA/2 spleen cells. Ia-antigen expression was determined using BALB/c anti-CKB serum and complement in a representative experiment. Con A sup- and Control sup-induced Ia-antigen expression were significantly different ($P < 0.01$) at dilutions <1:320.

Next it was determined whether the difference in the degree of cytotoxicity observed with the two antisera was a result of adsorption of soluble Ia antigen, or to an intrinsic difference in the potency of the two antisera to bind Ia-antigen or fix complement. The observation that BALB/c anti-CKB serum is reactive only to the I-A subregion, whereas A.TH anti-A.TL serum reacts with the entire I region favored the latter explanation. Con A sup and Control sup were prepared using C3H/HeN (Ia^k) spleen

TABLE I

*Comparison of Ia-Antigen Expression and DNA Synthesis by Cultured PEC**

Culture supernate	Dilution	Day 1		Day 3		Day 6	
		Mean cpm ± SEM‡	Ia^+§	Mean cpm ± SEM	Ia^+	Mean cpm ± SEM	Ia^+
			%		%		%
Media	—	155 ± 10	3.3	380 ± 29	0.5	252 ± 1	0.3
Con A sup	1:40	50 ± 5	4.0	1363 ± 158	11.6	1238 ± 137	23.5
Control sup	1:40	45 ± 9	2.0	1251 ± 207	1.0	1125 ± 279	1.2

* C3H/HeN PEC were cultured in enriched McCoy's 5-A medium with or without maximally active dilutions of Con A sup or Control sup prepared from DBA/2 mice. Duplicate cultures were prepared for assay on days 1, 3, and 6 of culture.

‡ One culture was pulsed for 6 h with 1 μC [^{3}H]TdR/well and the incorporation determined.

§ One culture was incubated with BALB/c anti-CKB serum and complement to determine the mean Ia-antigen expression.

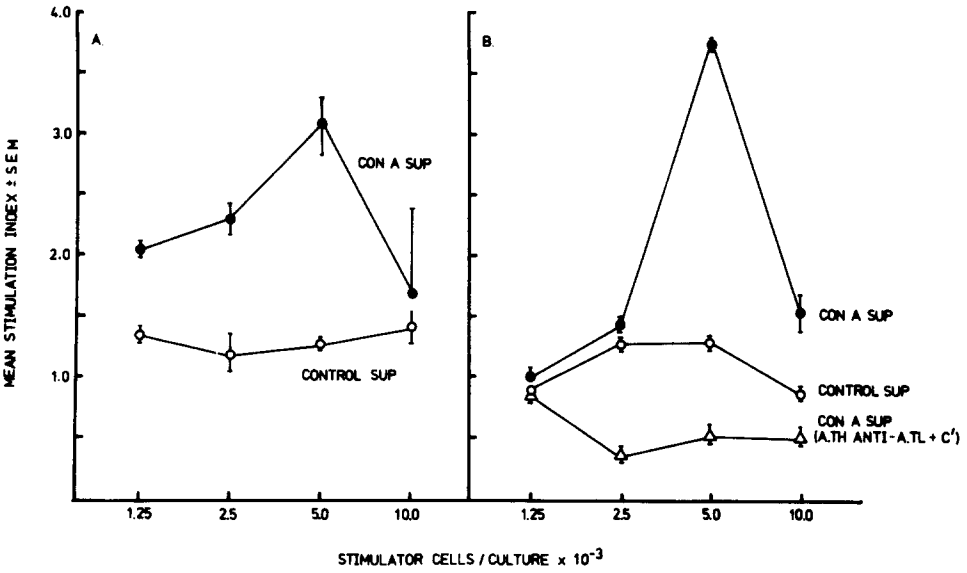

FIG. 5. Stimulatory capacity of Con A sup-incubated PEC in the MLR. Increasing numbers of C3H/HeN adherent PEC were incubated in a 1:80 dilution of Con A sup (●) or Control sup (○), prepared from DBA/2 spleen cells, for 5 d. The culture medium was then replaced with 1.6×10^5 DBA/2 splenic responder cells, and the cultures reincubated for 4 d. Proliferation was measured by [^{3}H]TdR incorporation during a 4-h pulse and is expressed as the mean stimulation index ± SEM in two representative experiments (A and B). The stimulation index is defined as the [^{3}H]TdR incorporation by mixtures of stimulator and responder cells divided by that of stimulator and responder cells cultured by themselves (~1,100 cpm in [A]; 1,499 cpm in [B]). In panel B Con A sup-incubated PEC were also treated with A.TH anti-A.TL serum and complement after initial incubation (△), and all cultures were washed three times before addition of responder cells.

cells, and were incubated with C3H/HeN (Ia^k) PEC. The Ia-antigen expression of Con A sup-incubated cultures, as determined with A.TH anti-A.TL serum was again higher than that using BALB/c anti-CKB serum (maximum CI of 44 and 28%, respectively, in a representative experiment), indicating an inherent difference in the cytotoxic potency of the two antisera, presumably a result of the subregion specificity of the latter antiserum.

Increased Ia Antigen Expression is a result of Conversion of Ia^- to Ia^+ PEC. To determine the lineage of macrophages that express Ia antigen in response to Con A sup, PEC were pretreated with A.TH anti-A.TL serum and complement (resulting in 7% cytotoxicity). These Ia^- PEC were subsequently cultured with DBA/2 (Ia^d) Con A sup or Control sup. for 7 d, and the percentage of Ia^+ cells determined using BALB/c anti-CKB serum and complement (Fig. 4). Pretreatment of the PEC with anti-Ia and complement failed to alter the subsequent dose-dependent conversion of Ia^- to Ia^+ PEC, arguing against the possibility that the induced Ia^+ PEC were generated by selective proliferation of preexisting Ia^+ macrophages. This experiment, however, does not rule out selective proliferation of Ia^+ PEC expressing insufficient antigen to be lysed by antiserum and complement.

It is possible that the Ia^- PEC which were converted to Ia^+ cells by incubation in Con A sup were either macrophage precursor cells undergoing exponential growth, as suggested by Lee and Wong (15, 22), or nondividing PEC. Therefore, the DNA synthetic rates of Con A sup- and Control sup-incubated PEC were determined by incubating C3H/HeN PEC with a maximally active supernate dilution prepared from DBA/2 mice. On days 1, 3, and 6 of culture replicate cultures were pulsed with [^{3}H]TdR and tested for Ia-antigen expression with BALB/c anti-CKB serum. The results of a representative experiment, shown in Table I, indicate that Con A sup- and Control sup-incubated PEC had virtually identical thymidine incorporation rates, although only Con A sup-treated cultures expressed Ia antigen. The cell densities and viabilities of Con A sup and Control sup-treated PEC were also similar. Furthermore, no MGF activity (21) was detectable in the Con A sup and Control sup. These data suggest that induction of Ia-antigen expression is not dependent on a proliferative expansion of an Ia^- precursor population.

Con A Sup-incubated PEC are Better MLR Stimulators than Control Sup-incubated PEC. Because the significance of Ia-antigen expression lies in the functional capabilities of Ia^+ cells, supernate-incubated PEC were tested in an Ia-dependent reaction, the MLR. Con sup-incubated PEC were cultured with allogeneic spleen cells in a MLR (Fig. 5); Control sup-incubated PEC were tested identically as a control. Con A sup-incubated PEC (24% Ia^+ in Fig. 5A; 14% Ia^+ in Fig. 5B with BALB/c anti-CKB serum) stimulated significantly greater proliferation of allogeneic spleen cells than Control sup-incubated cells (3% Ia^+ in Fig. 5A; 2% Ia^+ in Fig. 5B). The degree of proliferation was dependent on the number of PEC, with optimal stimulation consistently occurring with 5×10^3 PEC (3% of the responder cell number). Higher numbers of PEC had suppressive effects. Similar results were obtained when DBA/2 nylon wool-purified T-cells were used as the responder cells (data not shown).

Fig. 5B demonstrates the Ia-antigen dependence of the MLR. Duplicate Con A sup-incubated PEC cultures were prepared, one of which was treated with A.TH anti-A.TL serum and complement after Con A sup incubation. All cultures were washed three times, and the DBA/2 responder cells added. The data indicate that killing of the Con A sup-induced Ia^+ PEC and/or masking of the Ia-antigen by antibody abrogated their stimulatory capacity.

Discussion

Our data indicate that soluble factors produced by activated spleen cells induce Ia^- macrophages to express Ia antigens. When peritoneal macrophages were pre-

treated with anti-Ia and complement and subsequently incubated with Con A sup they still expressed Ia-antigens. This conversion of Ia^- to Ia^+ macrophages was, at least in part, a result of endogenous expression of Ia antigens, because a monoclonal antiserum reactive to the haplotype of the macrophages, but not that of the spleen cells from which the Con A sup was prepared, detected Con A sup-induced Ia-antigen expression. Determination of the MGF activity of the supernates, and comparison of the cell viabilities, densities and DNA synthetic rates of Con A sup- and Control sup-incubated cultures suggest that conversion of Ia^- to Ia^+ macrophages was not dependent on exponential proliferation. Conversion of Ia^- to Ia^+ macrophages by soluble factors may provide a means by which Ia^+ macrophages accumulate in the locality of a developing immune response, and therefore may account for the in vivo observations of Beller et al. (14). Conversely, the Ia-antigen expression by unstimulated peritoneal macrophages declined in culture. This observation supports the findings of Cowing and Dickler (2) and suggests that Ia^+ macrophages convert to Ia^- cells in the absence of regulatory factors.

Ia-antigen expression characterizes macrophages with the capacity to function as antigen-presenting cells. Therefore, we tested Con A sup-incubated macrophages for functional activity in an Ia-dependent reaction, the MLR (6–9). Con A sup-incubated macrophages stimulated significantly more proliferation of allogeneic spleen cells than Control sup-incubated cells. Additionally, treatment of Con A sup-cultured macrophages with anti-Ia and complement before the addition of responder cells abrogated their stimulatory capacity, confirming the Ia-dependence of the MLR.

A separate class of cells, dendritic cells, have been detected in the spleen (23), thymus (3), and to a much lesser extent, in the peritoneum (14), which can express Ia-antigens (3, 23) and induce an MLR. Because >95% of the cultured cells had the characteristic morphology of macrophages, and 97% of the cells actively phagocytosed latex particles, we conclude that in our cultures, macrophages rather than dendritic cells were induced to become functional Ia^+ accessory cells.

Although we have not yet identified the active material(s) within the Con A sup, preliminary results suggest that two possible sources of this activity can be eliminated. Bacterial endotoxin, a ubiquitous contaminant of FCS and culture media, is known to stimulate Ia-antigen expression of B lymphocytes (24). In preliminary experiments peritoneal macrophages incubated with a variety of doses of endotoxin failed to express Ia-antigens (data not shown). This indicates that the factor(s) present in the Con A sup that stimulated Ia-antigen expression was a product of spleen cells and not of microbial origin. CSF, detected by stimulation of bone marrow cells to produce macrophage/granulocyte colonies (25), has been detected in the Con A sup. Although CSF has been shown to have some macrophage stimulatory activities (26–28) as well as myeloproliferative effects (29), when we incubated peritoneal macrophages with an L-cell derived CSF preparation Ia-antigen expression was not increased (data not shown). Furthermore, long-term cultures of peritoneal-exudate macrophages in L-cell derived CSF resulted in macrophage colonies that were completely Ia^-.

Thus, activated spleen cells (most probably T lymphocytes) through the elaboration of a soluble factor(s) can induce both the phenotypic and functional conversion of Ia^- to Ia^+ macrophages. Parallel observations indicate that a lymphokine(s) can also induce Fc receptor expression in C3H/HeJ macrophages (17), and that a monocyte-derived mediator induces Lyt-1 lymphocytes to express Ia- antigen and antigen-

binding receptors (30). These observations suggest that nonspecific mediators can promote both genetically restricted and antigen-specific immunological reactions.

Summary

This investigation examined the effects of mediators derived from activated spleen cells on macrophage Ia-antigen expression and function. Incubation of adherent thioglycollate-induced murine peritoneal macrophages (>90% Ia^-) with concanavalin A (Con A)-stimulated spleen cell supernate (Con A sup) resulted in a dose-dependent increase in the percentage of Ia-containing (Ia^+) phagocytic cells, as detected by antiserum-and-complement-mediated cytotoxicity. The Ia-antigen expression of macrophages incubated with unstimulated spleen cell supernate supplemented with Con A (Control sup) declined. Pretreatment of the macrophages with anti-Ia and complement before addition of the Con A sup did not inhibit subsequent Ia-antigen expression, suggesting that Ia^- macrophages were converted to Ia^+ cells. These findings were not a result of adsorption of soluble Ia-antigen from the Con A sup, because Ia-antigen expression was detected by an antiserum specific for the haplotype of the macrophages but not that of the allogeneic spleen cells from which the supernate was prepared. Con A sup-cultured macrophages also stimulated the proliferation of allogeneic spleen cells significantly better than Control sup-cultured macrophages in the mixed leukocyte reaction (MLR). Pretreatment of Con A sup-cultured macrophages with anti-Ia and complement before addition of splenic responder cells abrogated their stimulatory capacity, indicating the Ia dependence of the MLR. We hypothesize that regulatory lymphokine(s) can induce both the expression of the Ia^+ phenotype by macrophages and the functional capability to stimulate the MLR, and that macrophages lose these capabilities in the absence of such mediator(s).

The authors thank Doctors Beda Stadler, Stefanie Vogel, and Sandra Smith-Gill for their assistance in the preparation of this manuscript. The authors also thank Sybil Ceja for excellent secretarial help.

Received for publication 16 July 1980.

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