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A B CELL SUBPOPULATION BINDS TO MACROPHAGES: THIS BINDING CAN BE DISRUPTED BY T CELLS[1]

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We have investigated the macrophage-binding properties of normal murine B cells and found that only a defined subpopulation of B cells can bind to macrophages. This subpopulation corresponds to the Lyb3-positive subpopulation. A population of T cells exists that disrupts this macrophage-B cell interaction. These findings are discussed in light of the demonstrated dependence on macrophages of several Lyb3+ cell functions.

Antigen-independent macrophage lymphocyte binding has been demonstrated in mouse (1–3), guinea pig (4–6), human (7), rabbit, and rat (8) systems. In the mouse it has been reported that thymocytes (2), lymph node and spleen cells (1), and some B cells (3) bind to macrophages. Given the functional interactions between macrophages and lymphocytes (reviewed in 9, 10), this *in vitro* demonstration of their propensity to associate is of considerable interest.

A number of B cell functions require macrophages or their products. These macrophage-dependent B cell functions include antibody responses to Type 2 thymus-independent antigens (T-I_2) (11, 12), proliferative responses to anti-IgM (13), dextran sulfate (14), sodium polyanethole sulfonate (15), and *in vitro* colony formation (16). Colony formation (17), the response to T-I_2 antigens (18, 19), and the response to anti-IgM (20) are known to be due to that specific subpopulation of B cells that is deficient in CBA/N (17) x-linked immunodeficient (*xid*)[2] (21) mice. Cells in this subpopulation bear the surface antigens Lyb3 (22) and Lyb5 (23). In addition, in mice with the H-2b haplotype the Lyb3-positive cells bear the antigen IaW39 (24, 25). Given the functional dependence of this subpopulation on macrophages, we were interested in examining the ability of these B cells to bind to macrophages.

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[1] This work was supported by National Institutes of Health Grants 1-Rol-CA 21348 and 5-Rol-CA 16172.
[2] Abbreviations used in this paper: Mϕ, macrophages; FlRaMFab, affinity-purified fluorescent rabbit anti-mouse Fab; BSA, bovine serum albumin fraction V; panned T cells, surface Ig-negative cells purified by the method of Wysocki and Sato (32); *xid*, x-linked immunodeficiency; 2ME, 2-mercaptoethanol; EDTA, ethylenediaminetetraacetic acid disodium; HEPES, *N*-2-hydroxyethylpiperazene-*N'*-2-ethanesulfonic acid; BAT, brain-associated thymus antigen; TRF, thymus-replacing factor.

MATERIALS AND METHODS

Mice. CBA/Tufts mice derived from a cross between CBA/Ca and CBA/J were maintained in our colony at Tufts. Except where otherwise noted these mice were used in all experiments. CBA/N mice were obtained from Dr. Harvey Cantor of Sidney Farber Cancer Center and maintained in our colony. (CBA/N × CBA/Tufts)F_1 and (CBA/N × BALB/c)F_1 were bred here.

The nude mice used were from the F_{5-8} generations of the 12th generation of backcrosses of the nude gene to CBA/Tufts mice. C57BL/6, BALB/c, and A/HeJ mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Mice ranged in age from 2 to 7 months. Within an experiment, all the mice used were either male or female.

Media. All cell incubations were done in RPMI 1640 (GIBCO) supplemented with 0.1% bovine serum albumin fraction V (BSA; Miles Laboratories) 290 μg/ml glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.02 M HEPES (see Abbreviations).

Macrophages. Noninduced macrophages were obtained by lavage of the peritoneal cavity. Thioglycollate-induced macrophages were obtained by the same procedure from mice that received an i.p. injection of 2 ml of 3% fluid thioglycollate medium (Difco) 4 days previously. Percent macrophages was calculated from the number of glass-adherent cells that ingested neutral red (26) or bacto-latex beads (Difco). Unless otherwise noted, the data presented were obtained by using induced macrophages, but as shown in *Results,* the major findings have been repeated by using cells obtained from lavage of a normal peritoneum.

Lymphocytes. Suspensions of thymus, lymph node, and spleen cells were made by gentle mincing through 100-mesh wire screen. Cells were treated with Tris ammonium chloride with 0.4% BSA (27) to remove red cells. Dead cells were removed by treatment of cell pellets with low ionic strength buffer according to the method of Von Boehmer and Shortman (28). The viability of the cells was tested by eosin dye exclusion before and after the test for binding. Both Tris ammonium chloride treatment and low-ionic buffer treatment were tested independently and found to have no effects on the parameters tested in this paper.

The binding assay. Five-eighths-inch cover glasses (discs), thinness 1 (Clay Adams), were individually washed in 90% ethanol, then dried and sterilized. One disc was then placed in the bottom of each well of a well tissue culture plate (Falcon 3008). 5×10^5 induced or 2×10^6 noninduced peritoneal cells were added in 1 ml to the wells and allowed to adhere overnight. After washing, the remaining adherent cells were 95 to 98% phagocytic. Suspensions of lymphocytes were then overlaid at 5×10^6/ml in each well, and the cultures were incubated for the indicated time. For the first 2 hr of incubation, the plates

were put at 37°C with 5% CO_2 in air on a Bellco rocker platform; the remaining incubations were performed without rocking. The nonbound cells were then removed by gently titrating the medium five times with a Pasteur pipette. This procedure was then repeated once with 1 ml of fresh medium. A consistent procedure for washing was vital for reproducible results. The viability of the nonbound cells was determined. For some experiments the viability of the bound cells was also determined by submerging the disc in 0.1% eosin. This ensured that differences between experimental groups was not a result of differences in viability.

A 0.05% solution of glutaraldehyde (Fisher Scientific) in Hanks' balanced salt solution (GIBCO) with 20 mM HEPES was added to the wells and left overnight. The fixed discs were washed with distilled water. Crystal violet (0.16%) was added to the wells for 2 min. The stain was removed, and the wells were washed two times with distilled water. The discs were removed from the wells (by manipulation with the bent bevel of a 27-gauge needle), dried, and mounted for observation.

Calculation of results. The slides were examined under a light microscope with a 40× objective. The macrophages along one diameter of each disc were counted with the aid of a micrometer disc. Each macrophage was scored as having 0, 1, 2, 3, or more bound lymphocytes. Macrophages with three or more lymphocytes bound were scored as positive. In several experiments the total number of bound lymphocytes was also counted. As shown in Figure 1, there is a direct correlation between the number of lymphocytes bound per hundred macrophages and percent of positive macrophages. The graphs in Figure 1 contain data obtained from macrophage-binding experiments done with spleen, thymus, anti-brain-associated thymus antigen- (BAT) resistant spleen cells or nylon wool-adherent cells as a source of lymphocytes. Since the data reported here deals specifically with macrophage-B cell binding, it should be pointed out that the values obtained with anti-BAT-resistant spleen cells or nylon wool-adherent cells fall on the regression lines shown with r = 0.89 for 2 hr co-incubation and r = 0.87 for 24 hr co-incubation. Counting the percent of positive macrophages had the advantage of being a faster method, and all data are expressed as such. The data presented were obtained by counting blind. Some experiments were counted independently by two observers, and similar results were obtained.

Cell separation. Cell fractionation on nylon wool columns was performed according to an adaptation of the methods of Julius *et al.* (29). Columns were run in media containing 1% BSA, and the effluent fraction was collected at the rate of approximately 50 drops per minute. The adherent fraction was removed by successively teasing the nylon wool with a forceps and compressing it with a syringe plunger.

Anti-BAT serum was prepared and characterized according to the method of Golub (30), and used with hamster complement (C) for the elimination of T cells. Surface Ig-positive cells were removed by the panning method (31) of Wysocki and Sato (32) by using affinity-purified goat anti-mouse Fab antibodies.

Anti-IaW39 serum was the gift of Dr. B. Huber and was used with rabbit C in cytotoxic assays according to the method previously described (24).

Enumeration of surface Ig-positive cells bound to macrophages. Three hundred microliters of a 1:100 dilution of affinity-purified fluoresceinated rabbit anti-mouse Fab (FlRaMFab; a gift from Dr. B. Huber) were added to wells containing the macrophage-bound cells. After 30 min on ice, 500 μl of fetal calf serum were overlaid. The discs were washed with medium, mounted wet (in 90% glycerol phosphate-buffered saline) and observed in the fluorescence microscope. Four hundred macrophage-bound lymphocytes were counted in each group, and the percent fluorescent cells was recorded.

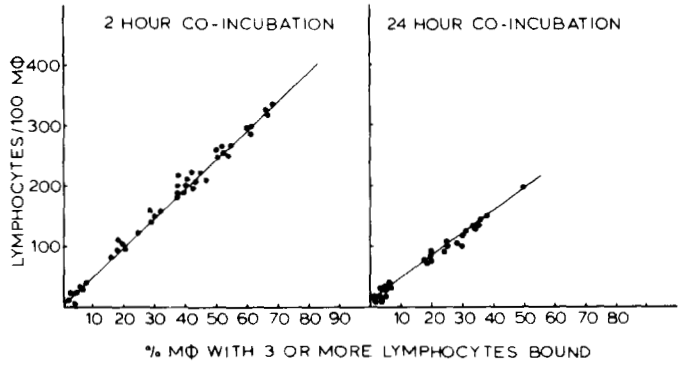

Figure 1. Correlation of per cent positive macrophages with number of lymphocytes/100 macrophages. Data represent individual counts from different experiments using syngeneic combinations of macrophages and lymphocytes from CBA/Tufts, C56BL/6, BALB/c, and A/HeJ mice. The lymphocytes were either of splenic or thymic origin. r = 0.89 for 2 hr co-incubation; r = 0.82 for 24 hr co-incubation.

RESULTS

B cell-macrophage binding in the mouse. Suspensions of lymphocytes were overlaid on macrophage monolayers. After 2 hr the nonbound cells were removed, and the remaining macrophage-bound cells were either stained with FlRaMFab or fixed and stained for observation. As shown in Figure 2, the vast majority of macrophage-binding cells are surface Ig-positive (B) cells. Spleen cells depleted of T cells (by treatment with anti-BAT and C) contained a large number of macrophage-binding surface Ig-positive cells. Spleen cells that were depleted of surface Ig-positive cells (by the panning method) contained a low frequency of macrophage-binding cells (Fig. 2). The binding of spleen cells to macrophages occurred in protein-free media and was inhibited by 2 mM EDTA (see Abbreviations) and 0.01% sodium azide (Table I).

The depletion of macrophage-binding cells. Since it was clear from the previous experiments that B cells could bind to macrophages, it was of interest to test whether all B cells share this property. This was done by incubating anti-BAT-resistant spleen cells on macrophage monolayers at a ratio of 1:1. The unbound cells were harvested, readjusted to 5×10^6/ml, and retested for binding. As shown in Figure 3, after two incubations on macrophage monolayers, the remaining cells did not bind to macrophages. Control cells that had been preincubated without macrophages bound as usual. This data suggests that there is

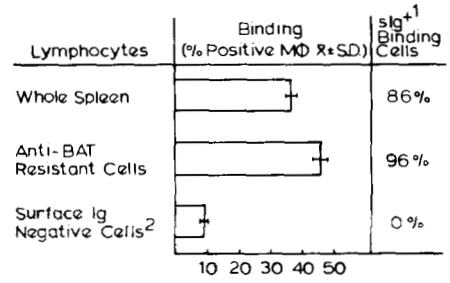

Figure 2. The binding of splenic T and B cells to Mϕ. 5×10^6 cells were incubated on macrophages for 2 hr. 1) As judged by FlRaMFab; 2) purified by the panning method of Wysocki and Sato (31).

TABLE I

Effect of various compounds on the binding of CBA/Tufts spleen cells to syngeneic Mϕ[a]

Inhibitor	Binding	Inhibition (Calculated from Mean)
	% positive Mϕ, $\bar{x}$ + S.D.	%
None	72 ± 5	
2 mM EDTA	7 ± 1	91
0.1% sodium azide	14 ± 5	81

[a] Binding observed after a 2 hr co-incubation of Mϕ and 5×10^6 whole spleen cells. Viability of nonbound cells did not vary significantly between groups (92 to 97%).

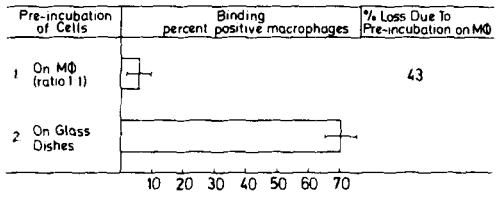

Figure 3. Nonbinding B cells can be purified from normal spleen by absorption of binding cells on macrophage monolayers. 1) Anti-BAT-resistant spleen cells were incubated on macrophage monolayers at a macrophage to lymphocyte ratio of 1:1 for 1 hr. The monolayers were then greatly washed to remove the nonbound cells. These harvested cells were incubated for a second time on macrophage monolayers, and the nonbound cells were harvested and tested for binding. 2) Anti-BAT-resistant spleen cells were incubated without macrophages present. By comparing the number of cells lost on macrophage monolayers to that lost on Petri dishes without macrophages, the percent of cells lost as a result of macrophage lymphocyte binding was calculated.

a subpopulation of B cells that binds to macrophages. It does not rule out, however, the possibility that all B cells can bind, but co-incubation leads to the loss of binding ability.

Purified nonbinding B cells are depleted of IaW39-positive cells. Fifty-five percent of anti-BAT-resistant spleen cells were removed by passage on macrophage monolayers. As a control, anti-BAT-resistant cells were also passaged on Petri dishes that did not contain macrophages. By subtracting the percentage of cells lost by passage without macrophage (12%) from that lost when macrophages were present (55%), the percent loss due to preincubation on macrophages was calculated (Fig. 3). From these numbers we estimate that between 43 to 55% of anti-BAT-resistant spleen cells can bind to macrophages. This percentage approximates the percentage of B cells that has been serologically defined as Lyb3-positive (22) Lyb5-positive (23), is recognized by anti-IaW39 serum (24), and is functionally defined by their ability to respond to T-I_2 antigens. If binder B cells constitute this same subpopulation of B cells, B cells depleted of binder cells should also be depleted of anti-W39-positive cells. As shown in Figure 4, this was the case. Normal anti-BAT-resistant C57BL/6 spleen cells contain a high frequency of both binding cells and IaW39-positive cells. Macrophage-passaged cells contained neither binding cells nor IaW39-positive cells. Since the IaW39 surface marker is an alloantigen selectively expressed on the Lyb3-positive subset of B cells in B6 mice (24, 25), these data show that macrophage-binding B cells are Lyb3 positive.

Binding of cells from mice with the xid defect. Anti-W39-sensitive, T-I_2-reactive B cells are absent in mice with x-linked recessive immunodeficiency (*xid*) (24). Since the previous experiment showed that anti-W39-sensitive cells contained within the binding subpopulation were not killed by anti-IaW39 plus C, one would predict that *xid*-defective mice would also have a binding cell defect. As shown in Figure 5, this was the case. Since this is an x-linked recessive defect, females from a (CBA/N × CBA/Tufts) cross are normal and males are defective. The normal F_1 females had binding cells both in whole spleen preparations and in the B-enriched nylon wool-adherent fraction. Few binding cells were observed among spleen cells from defective F_1 males. It should be pointed out that in both the previous experiment where B cells were depleted on macrophage monolayers and this experiment where the B cell *xid* defect was tested for binding, the proportion of B cells in the experimental populations was about one-third lower than in the control populations. However, it was shown independently that despite large decreases in the number of B cells overlaid, a significant level of binding was still observed. For instance, when 1×10^7 nylon wool-adherent cells were overlaid on macrophages, the observed binding was 54% positive macrophages after 2 hr and 39% positive macrophages after 24 hr. When 25×10^6 nylon wool-adherent cells were overlaid the observed binding was 32% positive macrophages after 2 hr and 26% positive macrophages after 24 hr.

Effect of T cells on macrophage-B cell binding. We fixed cultures of spleen cells and macrophages at various time points after the initiation of co-incubation and found that by 24 hr most of the lymphocytes have released from the macrophages.

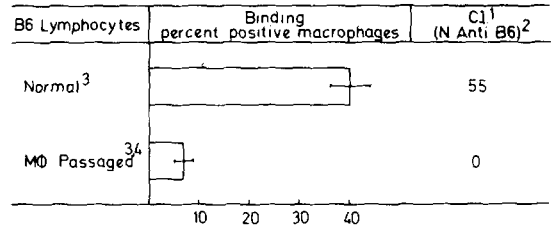

Figure 4. C57BL/6 (B6) spleen cells which had been depleted of macrophage-binding cells (by passage on macrophage monolayers) were depleted of cells bearing the IaW39 alloantigen recognized by N anti-B6 serum (24).

$$1)\ \text{C.I.} = \text{Cytotoxic index} \frac{\text{Experimental} - \text{control}}{100 - \text{control}} \times 100$$

2) CBA/N anti-B6 serum (24) defines the IaW39 alloantigen (25). 3) Anti-BAT-resistant spleen cells. Cells incubated on Petri dishes gave similar anti-BAT results (data not shown). 4) Macrophage-passage spleen cells were depleted of macrophage-binding cells according to the protocol described in Figure 3.

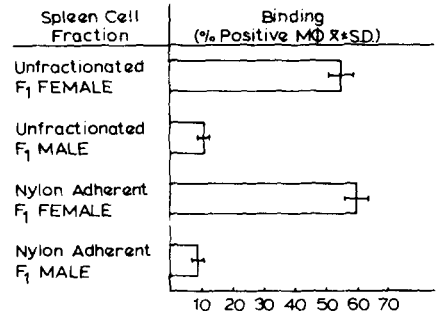

Figure 5. (CBA/N × CBA/Tufts)F_1 males have a macrophage-binding defect. 5×10^6 cells were incubated on macrophage monolayers for 2 hr. The nylon-adherent fraction refers to the B cell-enriched fraction (7% Thy 1 positive) from a nylon wool column. These experiments have been repeated by using (CBA/N × BALB/c)F_1 mice and by using anti-BAT-resistant spleen cells. The nylon wool-effluent fractions from both male and female spleens tested had a comparable (low) level of binding.

However, when this experiment was repeated with nylon wool-adherent or anti-BAT-resistant cells, the binding observed at 24 hr was always significantly higher than that observed with unfractionated spleen or lymph node cells (Table II). In other words, when T cells were removed from suspensions of spleen or lymph node cells, either by treatment with anti-BAT serum (data not shown) or by nylon wool fractionation (Fig. 6), macrophage-B cell binding persisted. The higher level of binding observed with T-depleted B cells compared with unfractionated cells could not be attributed solely to reduced numbers of B cells in the unfractionated cell preparation. After 24 hr, the binding observed in cultures that received 2×10^6 nylon wool-adherent cells was significantly higher than in those cultures that received 1×10^7 unfractionated spleen cells.

When peripheral T cells (purified either by the panning method or by nylon wool fractionation) were mixed with B cells, the binding occurred but did not persist (Fig. 6). The presence of T cells, therefore, can cause the release of B cells from macrophages. We have called this effect the remover cell effect and refer to the T cells that cause it as "remover cells."

This persistent binding of B cells and their release in the presence of T cells has also been demonstrated in the C57BL/6, A/HeJ, BALB/c strains of mice by using syngeneic combinations of cells (data not shown). Normal (noninduced) peritoneal exudate cells were also used as a source of macrophages (Table III). Although the noninduced macrophage monolayers bound more lymphocytes/macrophage, the basic findings were repeated. Nylon wool-adherent cells bind at a high level at both 2 and 24 hr. In the presence of nylon affluent T cells, the 24-hr binding is abrogated. These observations have been repeated with lymph nodes as a source of B and T cells and anti-BAT-resistant spleen cells as a source of B cells.

TABLE II

Binding of spleen and lymph node cells to Mϕ[a]

Expt. No.	Cells	Strain	Binding	
			2 hr	24 hr
			% positive Mϕ, $\bar{x}$ ± S.D.	
1	Lymph node[b]	CBA/Tufts	35 ± 4	4 ± 2
2	Lymph node[b]	CBA/Tufts	38 ± 4	7 ± 3
3	Spleen	CBA/Tufts	51 ± 5	10 ± 4
4	Spleen	C57BL/6	45 ± 2	3 ± 1
5	Spleen	CBA/Tufts	60 ± 4	6 ± 1
	Spleen	C57BL/6	57 ± 2	11 ± 1
	Spleen	BALB/c	60 ± 2	12 ± 3
	Spleen	A/HeJ	59 ± 7	8 ± 2

[a] Five × 10^6 cells were overlaid on macrophage monolayers. At the times indicated, the nonadherent cells were removed and the monolayers fixed.

[b] One × 10^7 lymph node cells were overlaid on the macrophage monolayer. C57BL/6 lymph node cells also gave comparable results.

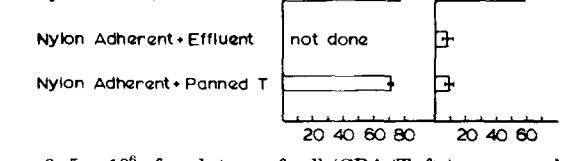

Figure 6. 5 × 10^6 of each type of cell (CBA/Tufts) were overlaid on macrophage monolayers. The spleen contains T "remover" cells which disrupt the binding that occurs between macrophage and B cells. This experiment has been repeated using anti-BAT-resistant spleen cells as a source of B cells with similar results.

TABLE III

Comparison of the binding of lymphocytes to thioglycollate-induced and noninduced Mϕ[a]

Spleen Fraction	Binding at 2 hr (% Positive Mϕ, $\bar{x}$ + S.D.)	
	Induced	Noninduced
Nylon wool effluent	18 ± 10	30 ± 11
Nylon wool adherent	66 ± 6	91 ± 8
Effluent + adherent (1:2)	73 ± 2	84 ± 6
Unfractionated	60 ± 3	80 ± 7
	Binding at 24 hr (% Positive Mϕ, $\bar{x}$ + S.D.)	
	Induced	Noninduced
Nylon wool effluent	5 ± 2	8 ± 4
Nylon wool adherent	16 ± 4	64 ± 3
Effluent + adherent (1:2)	7 ± 3	6 ± 3
Unfractionated	3 ± 1	5 ± 1

[a] The binding was observed when 5 × 10^6 cells are overlaid. The binding properties of induced and noninduced Mϕ were comparable.

DISCUSSION

We have shown that a surface Ig-positive, BAT-negative nylon wool-adherent B cell binds to macrophages. As reviewed in the introduction, macrophage requirements for a number of Lyb3-positive B cell functions have been demonstrated (11–16). The data presented here show that only this subpopulation of B cells binds to macrophages. Three lines of evidence support this conclusion: 1) mice with the *xid* defect have a binding cell defect, 2) purified nonbinding cells from normal mice are depleted of cells bearing IaW39, and 3) the percentage of B cells that bind equals the percentage that is IaW39 and Lyb3 positive. We have not ruled out the possibility that IaW39-positive cells become IaW39-negative as a result of interaction with macrophages during the purification procedure. However, since *xid* B cells do not bind to macrophages, we propose that normal adult B cells can be characterized as binding or nonbinding and that the binding population includes all IaW39-positive cells. Since the IaW39 surface marker is an alloantigen expressed only on the Lyb3-positive subset of B cells in $H2^b$-bearing mice (24, 25), we will refer to the macrophage-binding subpopulation of B cells in other strains tested as Lyb3 positive.

We have no data on the molecules involved in binding. It has been suggested that B cell surface Ig and Fc receptors on macrophages are involved.

In view of the macrophage-binding ability of this B cell subpopulation and the macrophage dependency of several functions of these B cells, it seems possible that direct contact with macrophages plays a role (either obligatory or accessory) in certain B cell functions. Macrophages may be required to present $T\text{-}I_2$ antigens to B cells in a manner analogous to presentation of antigens to T cells. In the case of antigen presentation by Ia-bearing macrophages to T cells, it is clear that whether T cells "see" antigen depends on the macrophage Ia (reviewed in 10). It is possible that $T\text{-}I_2$ antigen presentation also depends on a macrophage molecule. Even if this were not the case, antigen-independent macrophage-B cell binding could serve to promote contact between antigen-presenting and -recognizing cells.

The role of macrophages in polyclonal B cell proliferation is also unknown, but they may promote viability or provide a second signal. Garland and Owen (33) report that *in vitro* B cell colonies have a central "macrophage-like" cell. When B lymphocytes from CBA/N mice were placed in another *in vitro* colony-forming system, they failed to form colonies (16). As CBA/N mice lack macrophage-binding B cells, and colony formation is macrophage dependent, it is reasonable to propose that this is a macrophage-dependent B cell function that is restricted to the macrophage-binding B cell subpopulation.

Although the evidence argues for functional B cell-macrophage interactions, there have been reports that the macrophage requirement for some *in vitro* B cell functions can be circumvented in the presence of 2-mercaptoethanol (2ME). The addition of 2ME to macrophage-depleted cultures is necessary for a good response to DNP-Ficoll, a T-I_2 antigen (11), but it is still possible that there is an absolute macrophage requirement. Two reports suggest that 2ME and macrophages have synergistic effects on the response to T-I_2 antigens (34, 35). One report of a lack of a macrophage requirement in T-I_2 responses used a depletion protocol that does not affect the mitogenic response to PHA (36). This suggests that the depletion protocol was not efficient, since it is well documented that the T cell response to PHA is macrophage dependent (37).

Although B cell proliferation in response to anti-IgM (13), dextran sulfate (14), and sodium polyanethole sulfonate (15) requires macrophages, no such requirement exists in the presence of 2ME. A number of explanations for this finding have been proposed. 2ME may enhance the efficiency of the few contaminating macrophages (37). Alternatively, 2ME might activate an essential serum factor, and macrophages may have the same role (34). Whatever the role of 2ME, its ability to reduce or abolish a macrophage requirement does not tell us whether macrophages are normally required for the expression of a given function.

Supernatants from macrophage cultures can promote B cell colony formation *in vitro* (16) and responses to T-I_2 (35) antigens. These data argue against obligatory cell contact as the only mechanisms by which macrophages and lymphocytes can communicate. However, even in such situations where physiologic activities can be mediated by supernatant factors, these factors may function most efficiency via cell-cell contact. Macrophage-B cell binding may be a mechanism that ensures the proximity of the cells that are targets of soluble factors. Alternatively, factors may be composed of membrane products shed in culture, with cell interaction normally occurring via cell-cell contact. It may also be that contact with B cells provides a signal for macrophage-factor production or release.

Our results show that a T "remover" cell population can cause the release or removal of B cells from macrophage monolayers. Not all T cells can produce this effect, and remover T cells have no effect on thymocyte-macrophage binding (manuscript in preparation). Since the macrophages along the diameter of the disc were counted and no significant differences between groups in the number of macrophages was noted, we do not think it possible that the T cells are causing the release of macrophages and the B cells bound to them. Comparable data has also been obtained by using a technique based on the absolute numbers of B cells present rather than the percent of positive macrophages (manuscript in preparation). Therefore we can state that the presence of T cells does not result in a change in binding distribution (e.g., only two lymphocytes per macrophage). We therefore conclude that a T cell population exists that specifically affects the interaction of Lyb3-positive B cells with macrophages. Three published findings show that these particular B cells are indeed under the influence of T cells and/or their factors. 1) Parker *et al.* (38) have shown that, although B cells from normal mice proliferate and secrete Ig in the presenc of anti-Ig and supernatants from Con A-stimulated cultures, B cells from *xid* mice do not. 2) Ahmed and Scher (39) report that Lyb5-positive cells include the targets of the helper activity of thymus-replacing factor (TRF) in a primary antibody response to sheep red blood cells. 3) Scher *et al.* (40) found that the response of B cells from mice with *xid* defect could not be helped by activated T helper cells. In light of these data, we are currently investigating whether the remover cell is helper cell and whether T cell factors can influence macrophage-B cell binding.

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