THE ADJUVANT EFFECT OF CORYNEBACTERIUM PARVUM: T-CELL DEPENDENCE OF MACROPHAGE ACTIVATION*

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Several strains of anaerobic corynebacteria, when injected into mice as killed vaccines, have been shown to stimulate the phagocytic activity of the mononuclear phagocyte system (1, 2). The biological effects of one of these, Corynebacterium parvum, have been studied in particular and various reports indicate that macrophages are involved in many of the observed phenomena. These include stimulation of phagocytosis in vivo (3, 4), proliferation of macrophages in the liver (5-7), increased resistance to infections (8-10), antitumor activity in vivo (reviewed in 11) and in vitro (12, 13), depression of T-cell-mediated immunological reactions (10, 12, 14-16), and stimulation of antibody responses (17-19). It is believed that macrophages participating in these various phenomena are activated by treatment with C. parvum, but it is uncertain to what extent this activation is similar to the one described during infections with intracellular pathogens (20, 21) and particularly whether macrophage activation after C. parvum is mediated by T lymphocytes (22-24).

We reported recently that splenic macrophages from C. parvum-treated mice enhance the primary antibody response in vitro to sheep erythrocytes (SRBC) (19). Here we describe results of further experiments and provide evidence that macrophage activation by C. parvum in this system requires T lymphocytes.

Materials and Methods

Mice and Treatments. CBA female mice between 3 and 6 mo of age were used as donors of spleen and peritoneal cells. Experimental mice were pretreated with a single intravenous (i.v.) injection of C. parvum suspension (batch PX 289, The Wellcome Research Laboratories, Beckenham, Kent, England). Unless otherwise stated, all mice received 0.35 mg dry weight of organisms 5 days before their cells were harvested. Control mice were untreated. T-cell-depleted mice were produced by thymectomy at the age of 2-3 mo, followed by lethal whole body irradiation (800 R from a Siemens Stabilipan X-ray machine (Siemens-Reiniger-Werke AG, Erlangen, W. Germany) operating at 240 kV and 10 mA, with a target distance of 50 cm, 1 mm Cu filter, and a dose rate of 43.1 R/min) and reconstitution with 5 × 10⁶ syngeneic bone marrow cells given i.v. (TXB mice). Control mice were not thymectomized, but were irradiated and reconstituted with bone marrow (XB mice). These animals were used between 30 and 60 days.

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Abbreviations used in this paper: DNP-KLH, dinitrophenylated keyhole limpet hemocyanin; DNP-POL, dinitrophenylated polymerized flagellin; PFC, plaque-forming cells; TXB, thymectomized lethally irradiated bone marrow reconstituted mice; XB, lethally irradiated bone marrow reconstituted mice.

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after reconstitution. Some TXB mice were additionally reconstituted with $2 \times 10^6$ purified syngeneic T lymphocytes given i.v. 2 days before administration of C. parvum.

In one experiment mice that had been lethally irradiated (800 R) were reconstituted with $2 \times 10^6$ syngeneic spleen cells or purified T lymphocytes.

Unfractionated sea Kem 9 carrageenan (REX 7220, kindly provided by Dr. D. W. Renn, Marine Colloids, Inc., Rockland, Maine) was dissolved in sterile saline and 1 mg was administered to mice intraperitoneally 7 days before their spleens were removed for use in culture.

Mice were primed to keyhole limpet hemocyanin (KLH, Sigma Chemical Co., St. Louis, Mo.) by two intraperitoneal injections of 100 μg KLH and 100 μg ground freeze-dried human tubercule bacilli in 0.2 ml Alhydrogel (Miles Seravac Pty. Ltd., Maidenhead, Berkshire, England) given 10 wk apart. These mice were used at least 6 wk after the second injection.

Preparation and Fractionation of Cell Suspensions. Spleen cell suspensions were prepared in RPMI 1640 medium and fractionated into nonadherent and adherent cells as described before (19). Peritoneal cells were harvested by rinsing the peritoneal cavity of untreated or C. parvum-treated mice with 2 ml medium. Purified T lymphocytes were prepared from spleen cell suspensions using a scaled up version of the nylon wool column method (25). The cells eluted from the column contained 4-6% immunoglobulin-bearing cells compared to about 50% in unfractionated spleen cells (Dr. P. Kilshaw, personal communication). All cell separations were carried out under aseptic conditions.

The Antibody Response In Vitro. Spleen cells in complete RPMI 1640 medium, containing 5% heat-inactivated fetal calf serum and antibiotics (19), were cultured in Marbrook chambers (26) for 4 days, unless otherwise stated, at 37°C in humidified air containing 5% CO2. Cultures (1 ml) contained $2 \times 10^6$ unfractionated spleen cells or $13-14 \times 10^6$ nonadherent cells recovered after the removal of adherent cells (19). One million viable peritoneal cells or adherent spleen cells were added to some cultures. The cells were cultured in the presence of either $2 \times 10^6$ SRBC or 0.1 μg dinitrophenylated polymerized flagellin (DNP-POL, kindly provided by Dr. M. Feldmann, Department of Zoology, University College, London, England) as antigens. Dinitrophenylated keyhole limpet hemocyanin (DNP-KLH, kindly provided by Dr. A. Boylston, Department of Experimental Pathology, St. Mary's Hospital Medical School, London, England) was added in amounts of 0.1-10 μg/culture.

At the end of the culture period the cells were harvested, washed once, and resuspended in 1 ml medium. The antibody response to SRBC and DNP were measured in terms of direct plaque-forming cells (PFC) by the method of Cunningham and Szenberg (27), using untreated SRBC and SRBC treated with DNP-anti-SRBC Fab fragments (28), respectively. The results are given as means ± SE PFC per culture of quadruplicate cultures of cells pooled from several mice.

Results

The Antibody Response In Vitro of Spleen Cells from Mice Treated with C. parvum. The effect of varying doses of C. parvum given to mice on the antibody response of their spleen cells to SRBC in vitro is shown in Table I. Even after the smallest dose used (0.14 mg dry weight of organisms/mouse) the number of PFC was twice as high as in control cultures. Larger doses of C. parvum increased the PFC response up to 3.5 times over normal responses. In control experiments, not shown here, cells from treated mice cultured in the absence of SRBC gave numbers of PFC comparable to the normal background level (100 vs. 90).

The time-course of the in vitro response to SRBC of spleen cells from C. parvum-treated and control mice shown in Fig. 1 indicated that the enhancement could be observed throughout the period of culture and that the maximal PFC response occurred at the same time in both sets of cultures. In all other experiments the PFC response was measured, therefore, on day 4.

It has been reported that the time of C. parvum administration with respect to immunization can affect the antibody response in vivo (29, 30) and in vitro (18),
and both enhancement and depression have been observed. The results in Fig. 2 show that under the conditions of the present experiments the PFC response was enhanced when spleen cells were taken from mice between 2 h and 21 days after administration of *C. parvum*. The enhancement of the in vitro response was most marked 5-7 days after treatment and the interval of 5 days was selected for subsequent experiments.

In order to establish whether the enhancement of the in vitro antibody response of spleen cells from *C. parvum*-treated mice was limited to SRBC as antigen, an experiment was carried out using DNP-KLH, another T-cell-dependent antigen (31). The results given in Table II show that normal spleen cells responded poorly to DNP-KLH and that to obtain a good response the mice had to have been primed with the carrier KLH. Cells from *C. parvum*-treated animals gave, however, a response that was greater than the response of primed cells.

**Effect of Carrageenan on the Antibody Response.** The effect of carrageenan, a polysaccharide known to be toxic to macrophages (32), but not to lymphocytes (33), was studied in order to confirm the involvement of macrophages in the enhancement of the antibody response in vitro after administration of *C. parvum*. As can be seen from Table III, a single dose of carrageenan given 2 days before *C. parvum* markedly reduced the antibody response to SRBC. This was

### Table I

*Effect of Dose of *C. parvum* Administered to Mice on the Antibody Response of Spleen Cells In Vitro*

| Dose of *C. parvum* (mg) | Response to SRBC (PFC/culture) |
|--------------------------|---------------------------------|
| 0                        | 2,010 ± 107                     |
| 0.14                     | 4,436 ± 107                     |
| 0.175                    | 6,550 ± 153                     |
| 0.35                     | 6,400 ± 74                      |
| 0.70                     | 7,060 ± 134                     |
| 1.40                     | 7,200 ± 423                     |

![Fig. 1](image)

**Fig. 1.** The time-course of the in vitro response to SRBC of spleen cells from normal (●) and *C. parvum*-treated (○) mice.
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Fig. 2. The in vitro response to SRBC of spleen cells at different times after administration of *C. parvum*. The shaded area represents the response of normal cells.

**TABLE II**

*The Effect of Pretreatment with C. parvum on the In Vitro Response to DNP-KLH*

| Source of spleen cells | Dose of DNP-KLH (µg/culture) | PFC/culture |
|------------------------|-------------------------------|-------------|
| Normal                 | 0.1                           | 336 ± 98    |
|                        | 1.0                           | 162 ± 29    |
|                        | 10.0                          | 270 ± 31    |
| *C. parvum*            | 0.1                           | 2,506 ± 165 |
|                        | 1.0                           | 3,161 ± 349 |
|                        | 10.0                          | 2,212 ± 173 |
| KLH primed             | 0.1                           | 1,149 ± 123 |
|                        | 1.0                           | 2,131 ± 104 |
|                        | 10.0                          | 1,800 ± 163 |

the case with cultures containing unfractionated spleen cells as well as with cultures containing nonadherent cells from untreated mice and adherent spleen cells from animals pretreated with carrageenan.

**Requirement for T Cells in Macrophage Activation.** The dependence of macrophage activation by *C. parvum* on T lymphocytes was studied using cells from mice depleted of T cells by adult thymectomy and irradiation. The results of two experiments using SRBC as antigen and one using DNP-POL are given in Table IV. Unfractionated spleen cells from *C. parvum*-treated mice gave an enhanced response to SRBC, but not to DNP-POL. The response of cells from TXB mice to SRBC was depressed, while the response to DNP-POL was unaffected, indicating normal B-cell and defective T-cell function. Pretreatment with *C. parvum* of TXB spleen cell donors did not improve the deficient response to SRBC.

An enhanced response to SRBC, although somewhat smaller than that obtained with unfractionated spleen cells, was found in cultures containing nonad-
TABLE III
Effect of Pretreatment of Cell Donors with C. parvum and Carrageenan on the Antibody Response In Vitro

| Spleen cells in culture | Pretreatment of donors* | Response to SRBC (PFC/culture) |
|------------------------|-------------------------|-------------------------------|
| Unfractionated         | Nonadherent             | Adherent                      |
| +                      | -                       | -                             | +                      |
| + ‡                    | -                       | -                             | +‡                    |
| -                      | +                       | +                             | 5                     |
| -                      | +‡                      | +‡                            | 5‡                    |
| -                      | +‡                      | +‡                            | 5‡                    |

* Days before harvesting of cells.
‡ Cells from pretreated mice.

TABLE IV
Lack of Enhancement of the Antibody Response In Vitro by Macrophages from T-Cell-Depleted Mice Treated with C. parvum

| Spleen in culture | PFC/culture |
|------------------|-------------|
| Unfractionated   | Peritoneal cells |
| Nonadherent      | Anti-SRBC    |
| Adherent         | Anti-DNP     |
| Normal           | Exp. 1       | Exp. 2       |
| CP               | Normal Normal |
| TXB              | Normal CP    |
| TXB-CP           | Normal TXB-CP|
| Normal           | Normal XB    |
| Normal           | Normal XB-CP |

Mice used as cell donors are designated as follows: normal, untreated; CP, C. parvum-treated.

Heterent spleen cells which were supplemented with either peritoneal cells or adherent spleen cells from mice pretreated with C. parvum. We have described elsewhere (19) that in the reverse combination, i.e. normal adherent cells with C. parvum nonadherent cells, the response in vitro was not enhanced and have concluded that the enhancement is mediated by macrophages. However, when peritoneal or adherent spleen cells were obtained from C. parvum-treated TXB mice and used to supplement normal nonadherent spleen cells no enhancement of the antibody response was found and the numbers of PFC were similar to cultures that were supplemented with macrophages from either normal or TXB mice. On the other hand, macrophages from XB mice treated with C. parvum
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Table V
Recovery of the Ability of Macrophages from T-Cell-Depleted Mice Reconstituted with T Lymphocytes to Enhance the Antibody Response In Vitro

| Cells in culture          | Spleen                  | Peritoneal cells | Response to SRBC (PFC/culture) |
|---------------------------|-------------------------|------------------|--------------------------------|
| | Unfractionated | Nonadherent | Adherent | | |
| Normal | –             | –             | –             | 2,120 ± 97                     |
| CP     | –             | –             | –             | 5,986 ± 226                    |
| TXB    | –             | –             | –             | 376 ± 56                       |
| TXB-CP | –             | –             | –             | 420 ± 42                       |
| TXB-T  | –             | –             | –             | 1,990 ± 95                     |
| TXB-T-CP | –         | –             | –             | 4,750 ± 230                    |
| – Normal | Normal         | –             | –             | 1,710 ± 66                     |
| – Normal | –             | CP             | –             | 5,028 ± 128                    |
| – Normal | –             | TXB            | –             | 1,570 ± 93                     |
| – Normal | Normal         | –             | –             | 1,750 ± 51                     |
| – Normal | –             | TXB-CP         | –             | 1,766 ± 54                     |
| – Normal | –             | TXB-T          | –             | 3,920 ± 72                     |
| – Normal | Normal         | –             | Normal        | 1,720 ± 50                     |
| – Normal | Normal         | –             | CP             | 5,056 ± 175                    |
| – Normal | Normal         | –             | TXB            | 1,350 ± 66                     |
| – Normal | Normal         | –             | TXB-CP         | 1,410 ± 87                     |
| – Normal | Normal         | –             | TXB-T          | 1,906 ± 113                    |
| – Normal | Normal         | –             | TXB-T-CP       | 4,030 ± 210                    |
| TXB    | –             | Normal         | –             | 936 ± 90                       |
| TXB    | –             | –             | Normal         | 1,886 ± 50                     |
| – TXB  | Normal         | –             | –             | 670 ± 48                       |
| – TXB  | –             | Normal         | –             | 1,800 ± 45                     |

Mice used as cell donors are designated as follows: normal, untreated; CP, *C. parvum*-treated; T, reconstituted with purified T lymphocytes.

Did enhance the PFC response in vitro, although to a somewhat lesser degree than similar cells from normal *C. parvum*-treated animals.

Further evidence that activation of macrophages to enhance the antibody response in vitro requires T lymphocytes was obtained in the experiment in which TXB mice were reconstituted with purified T lymphocytes 2 days before administration of *C. parvum* (Table V). Spleen cells from TXB mice gave a much reduced response to SRBC, but the response was normal when cells from TXB mice reconstituted with T lymphocytes were used. Spleen cells from TXB mice treated with *C. parvum* failed to show an enhancement of the PFC response in contrast with the enhanced response of cells from TXB mice reconstituted with T lymphocytes. Similarly, peritoneal and adherent spleen cells from *C. parvum*-treated normal or TXB mice reconstituted with T cells, but not from TXB mice, enhanced the response of normal nonadherent spleen cells. The results shown at the bottom of Table V indicate that normal peritoneal cells, as opposed to adherent spleen cells, contained sufficient numbers of T lymphocytes.
TABLE VI
Role of Recruitment and Proliferation of Macrophages During Their Activation by C. parvum for the Enhancement of the Antibody Responses In Vitro

| Cells in culture | Peritoneal cells | Response to SRBC (PFC/culture) |
|------------------|-----------------|-----------------------------|
| Unfractionated   | Nonadherent     | Adherent                    |                             |
| Normal           | -               | -                           | 1,940 ± 44                  |
| C. parvum        | -               | -                           | 5,390 ± 142                 |
| Normal           | Normal          | -                           | 1,860 ± 108                 |
| Normal           | CP              | -                           | 4,066 ± 134                 |
| Normal           | X               | -                           | 1,540 ± 73                  |
| Normal           | X-CP            | -                           | 1,660 ± 147                 |
| Normal           | X-T             | -                           | 1,846 ± 80                  |
| Normal           | X-T-CP          | -                           | 3,676 ± 56                  |
| Normal           | X-S             | -                           | 1,536 ± 110                 |
| Normal           | X-S-CP          | -                           | 2,476 ± 204                 |
| Normal           | -               | Normal                      | 2,000 ± 86                  |
| Normal           | -               | CP                          | 4,830 ± 93                  |
| Normal           | -               | X                           | 1,776 ± 109                 |
| Normal           | -               | X-CP                        | 1,796 ± 123                 |
| Normal           | -               | X-T                         | 1,986 ± 163                 |
| Normal           | -               | X-T-CP                      | 2,560 ± 172                 |
| Normal           | -               | X-S                         | 1,796 ± 114                 |
| Normal           | -               | X-S-CP                      | 4,400 ± 226                 |

Mice used as cell donors are designated as follows: normal, untreated; CP, C. parvum-treated; X, irradiated; T, reconstituted with purified T lymphocytes; and S, reconstituted with spleen cells.

to restore the response of spleen cells from TXB mice to SRBC. This is in agreement with the fact that T lymphocytes are present among peritoneal cells (34).

Role of Recruitment and Proliferation in Macrophage Activation. It has been shown that C. parvum induces not only proliferation of macrophages resident in the liver, but also causes mobilization and proliferation of macrophage precursors from an extra-hepatic source (6). The C. parvum-induced macrophage proliferation was found to be largely responsible for the increase of phagocytic activity in vivo (7). In order to determine whether T-cell-dependent activation of macrophages after administration of C. parvum is dependent on their recruitment and proliferation the following experiment was performed. Mice received 800 R whole-body irradiation and within 6 h were reconstituted with either purified T lymphocytes or unfractionated spleen cells. These animals were treated with C. parvum 2 days later and their cells were harvested 7 days after irradiation. The adherent spleen cells or peritoneal cells were used to supplement nonadherent cells from untreated animals in culture vessels. The results showing the PFC response of various cell combinations are given in Table VI. It can be seen that macrophages from irradiated mice treated with C. parvum failed to enhance the PFC response, but that irradiation did not alter
the supporting function of macrophages from otherwise untreated animals. Reconstitution of irradiated mice with purified T lymphocytes restored the ability of macrophages to be activated by *C. parvum*. This restoration was complete in the case of splenic macrophages and partial in the case of peritoneal cells. Similar results were obtained with cells from mice reconstituted with spleen cells and treated with *C. parvum*, except that this time peritoneal cells enhanced the PFC response more than did adherent cells.

**Discussion**

The observations reported here confirm recent findings that the primary antibody response of spleen cells to SRBC in vitro is enhanced by pretreatment of mice with *C. parvum* (18, 19). This is in agreement with several reports on the adjuvant activity of this organism on antibody responses in vivo to this antigen (17, 29, 30, 35). The in vitro response to DNP-POL, a T-cell-independent antigen (36) was not, however, enhanced by pretreatment with *C. parvum*. This is in contrast to the findings that the in vivo response to other thymus-independent antigens such as pneumococcal polysaccharide (17) or DNP-levan (37), can be stimulated by *C. parvum*. The enhancement of the in vivo response to pneumococcal polysaccharide was dependent on the dose of antigen and the time of its administration. The first of these two factors did not play a role in the in vitro experiments, using doses of DNP-POL from 0.01 to 10 µg (unpublished data), and the reason for the discrepancy between the two systems is not known.

The enhancement of the in vitro response to SRBC in the present experiments is unique compared to other in vitro studies of adjuvant effects in which various materials were active either when added to cultures of unprimed spleen cells (38–41) or when added to cultures of cells from animals primed with the respective material (42, 43). In the case of *C. parvum*, cells from pretreated animals did not require additional exposure to the vaccine in order to give an enhanced response. On the other hand, addition of *C. parvum* to cultures of normal cells had no effect (unpublished results).

The present results, together with those reported earlier (19) show clearly that the adjuvant effect of *C. parvum* is mediated by adherent cells. The conclusion that these cells are macrophages is supported, in addition to their property of adherence, by the following observations: (a) they are radioresistant (19); (b) their function is suppressed after administration of carrageenan, which affects macrophages but not lymphocytes (32, 33, 44); (c) their function is depressed in late stages of infection with *Mycobacterium lepraemurium*, an obligate intracellular organism found in macrophages (45); (d) peritoneal cells, which presumably contain more T lymphocytes (34) than adherent spleen cells, were not better at enhancing the in vitro response. A similar enhancement of the antibody response to SRBC in vitro, which is also mediated by macrophages rather than lymphocytes, has been found using spleen cells from mice infected with intracellular bacteria, including *M. lepraemurium*,² BCG, and *Salmonella typhimurium* (unpublished results).

The way in which macrophages activated by *C. parvum* enhance the antibody response to T-cell-dependent antigens is uncertain at present. As macrophages

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² Sljivić, V. S., S. R. Watson, and I. N. Brown. Manuscript in preparation.
bind, ingest, and degrade antigens and present their immunogenic moiety to lymphocytes involved in antibody responses (reviewed in 46) it could be postulated that such handling of antigen may be modified in activated macrophages and result in enhanced responses. This is supported by the findings on the handling of KLH in vitro by peritoneal macrophages from C. parvum-treated mice (47). The present observation that the response to DNP-POL was not enhanced by C. parvum would not have contradicted this possibility in view of earlier reports that the in vitro response to DNP-POL is macrophage independent (48). It has been shown more recently, however, that in vitro responses to antigens, including POL, that had been considered to be macrophage independent, require adherent accessory cells (49). If this is the case, the present finding would indicate that C. parvum does not modify the interaction between macrophages and B lymphocytes during the response to DNP-POL. In addition, if antigen handling by macrophages is involved in the response to this antigen, the lack of enhancement after C. parvum would suggest that the handling of DNP-POL is not affected by C. parvum.

Another possibility concerning the mode of action of activated macrophages in the enhancement of the antibody response to SRBC is related to the findings that macrophage products can regulate the proliferation and function of lymphoid cells in vitro (50–52). If it is to be postulated that the enhancement by C. parvum is mediated by some similar factor, this will have to act on T lymphocytes, since the response to DNP-POL was unaffected. This conclusion finds some support in the evidence that macrophages are required for the generation of helper T cells in vitro (53) and that factors released from macrophages are also active (54). It is possible that macrophages activated by C. parvum are more effective in the induction of helper cells in the presence of antigen, which would explain the lack of enhancement of the response to T-cell-independent antigens, such as DNP-POL. The finding that spleen cells from C. parvum-treated mice gave equally good, if not better, responses to DNP-KLH, as compared to cells from KLH-primed animals, provides additional evidence that C. parvum can nonspecifically increase T-cell helper function. It remains to be established whether this too is mediated via macrophages.

As distinct from the role of T lymphocytes in the expression of the function of activated macrophages, the present results show that T lymphocytes are required for the activation of macrophages by C. parvum. This is in general agreement with the requirement for T cells in the action of other adjuvants (43, 55) and, more specifically, with the role of T cells in the activation of macrophages during the course of infection with intracellular pathogens, such as Mycobacteria, Brucella, Salmonella, and Listeria (20, 21). However, administration of C. parvum has been reported on the one hand to suppress cell-mediated reactions and T-cell function and, on the other, to stimulate certain macrophage functions in the absence of T lymphocytes. Thus, C. parvum suppressed or reduced delayed hypersensitivity reactions during the course of Salmonella infection (10) and after administration of antigens in vivo (56). In addition, various reactions involving splenic T lymphocytes, such as the mixed lymphocyte and graft vs. host reactions, as well as the response to phytohemagglutinin have been shown to be impaired after intravenous injections of large
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(14), but not small doses of C. parvum (57) and this suppression was found to be mediated by macrophages (15).

Administration of C. parvum by the subcutaneous route gives rise to a significant level of delayed hypersensitivity to the organism within 3 days, which reaches peak on day 6 (58–60). This kinetics closely parallels the enhancement of the antibody response to SRBC in the present experiments and supports the view that activated macrophages which enhance this response arise through a delayed hypersensitivity reaction to C. parvum. Peritoneal cells from C. parvum-treated mice were found to be as effective as spleen macrophages in enhancing the antibody response in the present experiments. In view of the negligible localization of C. parvum in the peritoneal cavity after i.v. administration (61) it is possible that peritoneal macrophages are activated through interaction with sensitized circulating T lymphocytes. The role of T cells in the activation of macrophages by C. parvum is also illustrated by the work of Christie and Bomford (13). In their experiments activated macrophages, which inhibited DNA synthesis of leukemia cells, could only be generated when C. parvum-sensitized, rather than normal, cells were cultured with C. parvum. The activation was abolished by pretreatment of sensitized cells with anti-0 serum. However, in in vivo experiments C. parvum was almost equally effective in TXB and XB mice, suggesting either the stimulation of residual T cells or a mechanism of macrophage activation which is independent on T lymphocytes (24). An apparent independence of stimulation of various macrophage functions on T lymphocytes has been reported using different experimental models. These include stimulation of phagocytic activity in T-cell-deprived mice (22), generation of macrophages cytotoxic to tumor cells in both these and nude mice (23), and a relative increase in resistance to Listeria infection in nude mice (62). It thus appears that certain macrophage functions can be stimulated by C. parvum in vivo in the absence of T cells, in contrast to the present results which clearly indicate that activation of macrophages capable of enhancing the antibody response is T-cell dependent.

Summary

Splenic and peritoneal macrophages from mice treated with Corynebacterium parvum enhanced the antibody response in vitro of normal nonadherent spleen cells to SRBC, but not to DNP-POL. This enhancement was dependent on the dose and time of administration of C. parvum and could be abrogated by pretreatment with carrageenan. Macrophages from T-cell-depleted mice failed to enhance the response, but this ability was restored if the mice had been reconstituted with purified T lymphocytes. Macrophages that are activated by C. parvum are a resident nondividing population. It is postulated that activated macrophages, capable of enhancing antibody responses to T-cell-dependent antigens, arise through a cell-mediated reaction to C. parvum.

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