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Effect of *Bacillus Calmette-Guérin* on the *in vitro* generation of cytotoxic T lymphocytes

II. ROLE OF INTERLEUKIN-1-LIKE FACTORS AND OF SOLUBLE SUPPRESSOR FACTORS

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Accepted for publication 14 May 1985

Summary. The injection of BCG vaccine in C57BL/6J mice results in the suppression of the generation of cytotoxic T lymphocytes (CTL) in mixed lymphocyte cultures (MLC) and of mitogenic rections to concanavalin A (Con A). Suppression is mediated by macrophage-like suppressor cells. Since previous work had indicated that suppression involved the inhibition of the production of interleukin-2 (IL-2), the effects of BCG on interleukin-1 (IL-1), a monokine required for IL-2 production, were investigated. It was found that the release of IL-1-like activity in spleen cell cultures stimulated with LPS or Con A was increased by previous BCG treatment of the cell donors. In MLC, the release of IL-l-like activity was also increased by BCG. However, the detection of IL-l-like activity in MLC supernatants was prevented by the presence of a suppressor factor. In this case, the IL-l-like activity could be separated with gel filtration from the suppressor factor which had higher molecular weight. The production of IL-l-like activity by CBA/J spleen cells, which are not suppressed by BCG, was not significantly different from that of C57BL/6J cells, which are markedly suppressed. Moreover, the addition of IL-1 to the BCG-suppressed cultures not only did not restore normal reactivity, but actually further suppressed CTL formation. It was concluded that BCG-induced suppression cannot be attributed to decreased IL-1 activity. The suppressor factor discovered during these investigtions may have a role in this type of suppression.

Abbreviations: BCG, *Bacillus Calmette-Guérin*; CMC, cell-mediated cytotoxicity; Con A, concanavalin A; CTL, cytotoxic T lymphocytes; IL-1, interleukin-1; IL-2, interleukin-2; LPS, *E. coli* lipopolysaccharide; MLC, mixed lymphocyte culture(s).

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INTRODUCTION

The injection of *Bacillus Calmette-Guérin* (BCG) into mice of certain strains results in a marked immunosuppression which is apparently mediated by macrophage-like suppressor cells (Klimpel & Henney, 1978; Schrier, Allen & Moore, 1980; Castes *et al.*, 1981; Turcotte, 1981). In a previous report (Kendall & Sabbadini, 1981), we demonstrated that BCG, injected intravenously in C57BL/6J mice, induced suppression of the cell-mediated cytotoxicity (CMC) developed in mixed lymphocyte cultures (MLC) against allogenic targets. Spleen cell populations from BCG-treated mice contained nearly normal numbers of precursors of cytotoxic T lymphocytes (CTL). However, the production of non-specific helper factor(s) possessing characteristics similar to those of interleukin-2 (IL-2)

was suppressed. Since such helper factor(s) appeared to be necessary for a normal reaction, and since its (their) addition to the cultures reconstituted a nearly normal response of BCG-suppressed spleen cells, it was concluded that the lack of IL-2-like factor(s) was the major cause of the suppression induced by BCG.

Given that IL-2 is produced by Lyt-1 positive cells which respond to allogeneic Ia determinants (Wagner *et al.*, 1980) and, concomitantly, to IL-1 (Mizel, 1982), such a suppression of IL-2-like activity could have been attributed to the inhibition of Lyt-1 positive cells, probably mediated by macrophage-like suppressor cells. Alternatively, the suppressed IL-2-like activity could have been due to an interference of suppressor cells or suppressor factors with the action of IL-1, or to a decreased production of IL-1. This last mechanism might have been considered unlikely, since other investigations (Mitchell *et al.*, 1973) had shown increased IL-1-like activity after treatment with BCG. However, these authors had used strains of mice in which BCG potentiates immune responses rather than suppressing them. It is known that responses to BCG are under genetic control (Schrier *et al.*, 1982) and vary from strain to strain. Therefore, we decided to investigate the effects of BCG on IL-1 in C57B/6J mice. This led to the observations reported here which demonstrate the production of high levels of IL-1 and, at the same time, of suppressor factors by spleen cells from BCG-treated mice. Such suppressor factors may be involved in the inhibition of IL-2 production and in the consequent suppression of CMC.

MATERIALS AND METHODS

Animals

Female mice of the strains C57BL/6J (H-2^b, Igh^b), CBA/J (H-2^k, Igh^j), BDF_1/J (H-2^b/H-2^d), hybrids of C57BL/6J and DBA/2J and C3H/HeJ were obtained from the Jackson Laboratory, Bar Harbor, ME. All animals were 6–8 weeks old at the time of BCG inoculation.

Materials

Freeze-dried BCG vaccine (Connaught Medical Research Laboratories, Toronto, Canada) containing $1–3 \times 10^7$ viable units/mg was reconstituted in PBS and injected i.v. The animals received a single injection of 500 μg and were killed 2 weeks later. Con A was obtained from Sigma (St Louis, MO) and used at the concentration of 1 μg/ml. Purified human IL-1 was obtained from Genzyme, Norwalk, CT. The purity of the preparation was verified by polyacrylamide gel electrophoresis, which demonstrated a single protein band with apparent molecular weight consistent with that of IL-1 (Mizel, 1982); the activity of this preparation was confirmed in the thymocyte assay described below. Lipopolysaccharide *E. coli* 0111-B4 (LPS) was obtained from Difco Laboratories, Detroit, MI.

MLC-CMC

Mixed cultures of C57BL/6J responder spleen cells and irradiated (1500 rads) BDF_1 stimulator spleen cells were started 2 weeks after the injection of BCG to the donors of the responder cells. For the separation of adherent from non-adherent cells, spleen cell populations were incubated for 1 hr in plastic tissue culture petri-dishes (Falcon Plastics, Oxnard, CA). The loose cells were resuspended by rocking the plates, and were subjected to a second incubation under the same conditions. The adherent cells obtained in the first incubation were recovered by scraping the plates with a rubber policeman.

The cultures (six replicates of each) were carried out in tissue culture plates with U-bottomed wells (Limbro plates, obtained from Flow Laboratories, McLean, VA) containing a total volume of 0·2 ml of culture medium (RPMI-1640 medium supplemented with 5% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin). The number of responder cells varied as specified for each experiment, and the number of stimulator cells was kept constant at 2×10^5 per well. The cultures were incubated in a humidified atmosphere of air with 5% CO_2 for 4 days. At the end of this incubation period, 0·1 ml of a supernatant was withdrawn from each culture without disturbing the sedimented cells. An equal volume of 0·1 ml of suspension containing 2×10^4 P815 $\times$ 2 cells (of DBA/2J origin), labelled with ^{51}Cr as previously described, was then added in such a way as to produce enough mixing in the well to resuspend the cultured cells. The plates were then centrifuged for 5 min at 400 ***g*** and incubated for 4 hr. At the end of this second incubation period, 0·1 ml of the supernatant was withdrawn to measure the amount of radioactive chromium released.

The percentage lysis was calculated from the formula

$$\frac{(E-S)}{(T-S)} \times 100$$

where E = c.p.m. in the supernatant of the experimen-

tal well, T = total releasable radioactivity (i.e. mean c.p.m. in the supernatants of wells in which target cell lysis had been induced with distilled water containing 0·001% sodium dodecyl sulphate), and S = spontaneous release (i.e. mean c.p.m. in the supernatant of wells in which the target cells had been incubated with tissue culture medium alone). Spontaneous release is not shown with the results of the individual experiments reported here. It varied from 8% to 12% in the 4-hr incubation period used for these experiments. Any experiment in which spontaneous release exceeded these values was considered invalid and was not included in these results. Moreover, controls in which the responder and the stimulator cells were not mixed were routinely carried out. Any experiment in which ^{51}Cr release in these controls significantly exceeded spontaneous release was also considered invalid.

Production of active supernatants

IL-1 production was stimulated with LPS (5 μg/ml) or Con A (1 μg/ml) in 20-ml cultures containing 10^7 cells/ml. Cultures were carried out for 48 h in tissue culture flasks kept in the upright position. MLC stimulation for the same purpose was carried out for 40 h (Farrar, Koopman & Fuller-Bonar, 1977) with a 1:1 ratio of stimulator to responder cells with a final cell concentration of 15×10^7/ml. The supernatants were collected by centrifugation and were dialysed against tissue culture medium.

Gel filtration

Tissue culture supernatants were concentrated five to ten times with pressure dialysis and dialysed against 0·05 M phosphate buffer, pH 7·4, containing 0·1 M NaCl. Five-ml samples were applied to a $2{\cdot}6 \times 70$ cm Sephacryl S200 column (Pharmacie Fine Chemicals, Uppsala, Sweden) equilibrated with the same buffer. Before testing for biological activity, fractions were dialysed against tissue culture medium. Fetal calf serum was then added to a final concentration of 5%.

Thymocyte assay

IL-1 activity was measured as the capacity to increase the uptake of [^{3}H]TdR by Con A-stimulated thymocytes (Gery, Gershon & Waksman, 1971). C3H thymus cells (5×10^5 per culture) were stimulated with Con A at a final concentration of 1 μg/ml in the same medium as for the MLC-CMC assay. In each culture, 0·1 ml of medium containing thymus cells and Con A was mixed with 0·1 ml of the culture supernatant to be assayed, serially diluted with culture medium. Controls consisted of culture medium containing the same IL-1 inducer (LPS or Con A) as the supernatant, also in serial dilution. Fractions obtained by gel filtration were dialysed against culture medium before adding fetal calf serum to the final 5% concentration and used in serial dilution in the assay. The cultures were incubated for 3 days, and then 0·5 μCi of [^{3}H]TdR was added to each culture. The plates were further incubated overnight before collecting the cells on fiberglass filters with a cell harvester (Skatron AS, Lierbyen, Norway). The radioactivity incorporated by the thymus cells was measured by liquid scintillation.

Assay for IL-2 activity

Culture supernatants suspected to contain IL-2 activity were added to a long-term CTL line (2×10^4 cells per culture) produced in this laboratory and shown to be IL-2 dependent. The cells were exposed to various dilutions of the supernatants. Cell proliferation was measured with the [^{3}H]TdR assay and CMC was measured with the ^{51}Cr assay after 24 h in culture.

Statistical methods

Significance of the differences among groups in each experiment was estimated with one-way analysis of variance. Standard deviations for each group are indicated in the tables and figures.

RESULTS

Demonstration of plastic-adherent suppressor cells

In order to confirm the finding of adherent suppressor cells reported by Klimpel & Henney (1978), normal spleen cells were mixed with spleen cells from BCG-treated donors. The BCG-spleen cells were either unfractionated or separated into adherent and non-adherent cells. The unfractionated BCG-spleen cells produced almost no response (Table 1), the adherent cells did not respond at all, while the non-adherent cells developed a significant, but still lower than normal, response. When BCG-spleen cells were mixed with normal spleen cells, they demonstrated a marked suppressive activity that was entirely contained in the adherent fraction.

The Con A reactivity of spleen and lymph node cells (measured by [^{3}H]TdR incorporation) was also suppressed by prior BCG treatment and was sensitive to the suppressor activity of adherent spleen cells from BCG-treated donors (results not shown). The nature

Table 1. Suppressor effects of adherent and non-adherent spleen cells from BCG-treated donors

Normal spleen cells*	No. ($\times 10^{-4}$) and type of BCG-spleen cells	% lysis (±SD)
None	3·3 Unfractionated	3·3±0·5
	10 Unfractionated	4·8±0·9
	3·3 Adherent	0†
	10 Adherent	0†
	3·3 Non-adherent	18·5±1·6‡
	10 Non-adherent	32·0±2·9‡
10^5	0	56·7±3·9
	3·3 Unfractionated	28·0±3·1‡
	10 Unfractionated	21·3±4·2‡
	3·3 Adherent	16·5±2·2‡
	10 Adherent	9·6±1·1‡
	3·3 Non-adherent	54·9±3·5†
	10 Non-adherent	66·3±4·3‡

* Normal C57BL/J6 spleen cells were mixed with unfractionated, adherent or non-adherent C57BL/6J BCG-spleen cells and with 2×10^5 mitomycin-treated BDF_1 stimulator cells. The CMC assay was carried out 4 days later.

† Not significant.

‡ Significantly different ($P < 0.01$) from the respective controls.

of such adherent suppressor cells was not further investigated since several authors have concluded that these are macrophages or macrophage-like cells (Klimpel & Henney, 1978; Schrier *et al.*, 1980).

Production of IL-1-like factors by BCG-spleen cells

In order to establish whether or not the suppression of CMC by BCG involved an alteration of IL-1 activity, spleen cell cultures were stimulated with LPS and with Con A, both good IL-1 inducers (Mizel, 1982). Since culture supernatants from BCG-treated donors were shown to contain low molecular weight suppressor factors (L. Mellow and E. Sabbadini, unpublished observations), the supernatants in this and in all subsequent experiments were dialysed before the assay. IL-1-like activity was observed after stimulation with LPS (Fig. 1a) and Con A (Fig. 1b). In both cases, the activity released by BCG-spleen cells was significantly higher ($P < 0.01$) than that of normal supernatants. Tissue culture media containing LPS or Con A in similar concentrations had no significant effects. Both the normal and BCG supernatant were found to be devoid of IL-2 activity (results not shown in detail here).

To support the IL-1 nature of the amplifier factor, the supernatants of the LPS- and Con A-stimulated cultures were subjected to gel filtration through a Sephacryl S200 column. The amplifier activity of both normal and BCG supernatants was contained in fractions with apparent molecular weight in the 14,000–20,000 range, which is consistent with that of IL-1. The suppression induced by Sephacryl fractions from BCG-spleen cell cultures was somewhat higher than that from normal cultures. These results (not

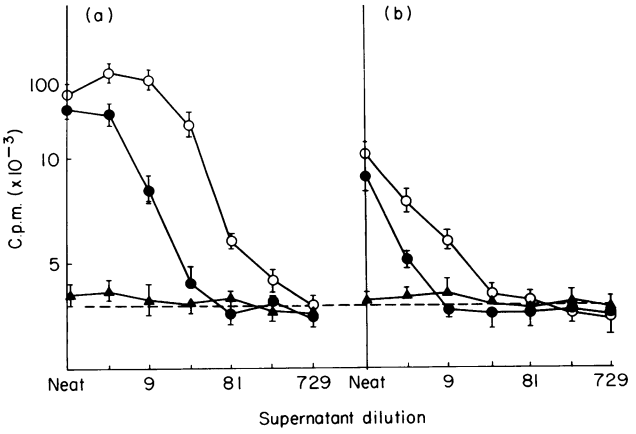

Figure 1. The dialysed supernatants of (a) LPS- and (b) Con A-stimulated cultures of spleen cells from (●) normal and (○) BCG-treated C57BL/6J donors were added in various dilutions to cultures of C3H thymus cells stimulated with Con A. Tissue culture medium containing either LPS or Con A was used in control cultures (▲). The control value with tissue culture medium only is represented by the broken horizontal line.

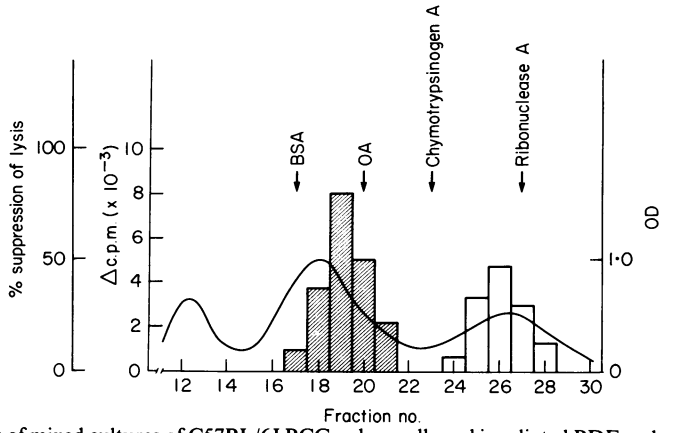

Figure 2. The supernatant of mixed cultures of C57BL/6J BCG-spleen cells and irradiated BDF_1 spleen cells was fractionated in a Sephacryl S200 column. The continuous line represents optical density; (□) increase, over control values, of tritiated thymidine incorporation (▲ c.p.m.) in cultures of Con A-stimulated C3H thymocytes to which the various fractions were added; (■) percentage suppression of tritiated thymidine incorporation in MLCs to which the various fractions were added.

shown in detail) were similar to what is presented below for MLC supernatants (Fig. 2).

The search for IL-1 activity in MLC supernatants at first gave ambiguous results. MLC supernatants from either normal or BCG-spleen cell cultures sporadically demonstrated low activity, while in most of the cases no activity at all was detected. Attempts to increase the concentration of IL-1 by precipitation with 60% saturated ammonium sulphate (Mizel, 1982) followed by reconstitution of the precipitated material in volumes 10–20 times smaller than the original, increased the activity only marginally and only in some experiments. On the other hand, IL-1 activity was clearly detectable after gel filtration on Sephacryl S200 (Fig. 2). When detected in this way, the IL-1 activity of supernatants of BCG-spleen cell cultures was usually higher than that of normal cultures (results not shown in detail here).

Detection of suppressor factors in MLC supernatants

The observation that IL-1 activity was more easily detectable in the appropriate gel filtration fractions than in whole supernatants suggested that some suppressor factor may have been removed in the fractionation procedure. The different gel filtration fractions were, therefore, tested for the capacity to suppress the MLC ($[^3H]$TdR incorporation assay) and the CMC (^{51}Cr assay) reactions of normal C57BL/6 spleen cells stimulated with irradiated BDF_1 cells. The results reported for the MLC reaction in Fig. 2 demonstrate a significant suppressor activity of fractions with an apparent molecular weight between those of bovine serum albumin and ovalbumin. Thus, gel filtration revealed the simultaneous presence of an IL-1-like stimulator factor and of a suppressor factor in supernatants of cultures of BCG-spleen cells stimulated with allogeneic cells. The presence of contrasting activities had prevented the detection of either of them in the unfractionated supernatant.

IL-1 production in CBA mice

The response to BCG may vary from suppression to potentiation of immune responses in various strains of mice. This different responsiveness appears to be controlled by genes linked to the immunoglobulin heavy chain (Igh) complex (Schrier *et al.*, 1982). Thus, the C57BL/6J strain (Igh^b) is markedly suppressed, while the CBA/J strain (Igh^j) presents, after BCG treatment, either normal or potentiated immune responses. These findings were confirmed in our laboratory (L. Mellow and E. Sabbadini, unpublished observations). If IL-1 were in any way involved in the mechanism of suppression, one would expect to observe major differences in IL-1 production between the CBA/J and the C57BL/6J strains with treatment with BCG. As shown in Table 2, CBA/J and C57BL/6J spleen cells, after BCG treatment, produced comparable amounts of IL-1-like activity upon stimulation with LPS, Con A or alloantigens. IL-2 activity was not found in the same fractions. Suppressor activity of the type shown in Fig. 2 in C57BL/6J supernatants was not found in CBA/J supernatants.

Table 2. Costimulatory activity in the thymocyte assay of gel filtration fractions* of supernatants of LPS-, Con A- and alloantigen-stimulated cultures of C57BL/6J and CBA/J spleen cells

Responsive cells†	Stimulator	Pretreatment of donors	Costimulatory activity‡
CBA/J	LPS	None	1:32
	LPS	BCG	1:148
	Con A	None	1:16
	Con A	BCG	1:94
	Alloantigenic C.	None	NM§
	Alloantigenic C.	BCG	1:4
C57BL/6J	LPS	None	1:42
	LPS	BCG	1:164
	Con A	None	1:20
	Con A	BCG	1:86
	Alloantigenic C.	None	NM
	Alloantigenic C.	BCG	1:3

* Supernatants of stimulated and unstimulated cultures were subjected to gel filtration in Sephadex G200 columns under conditions otherwise identical to those described for Sephacryl columns. Fractions with apparent molecular weights of 12,000–19,000 were pooled and assayed for IL-1-like activity.

† Animals of CBA/J and C57BL/6J strains were either untreated or injected with 500 μg BCG 2 weeks before killing.

‡ IL-1-like activity is expressed as the maximal dilution capable of inducing a doubling of the c.p.m. as compared to the control cultures (1422 ± 125). Control supernatants with no stimulators had no measurable activity.

§ NM, not measurable.

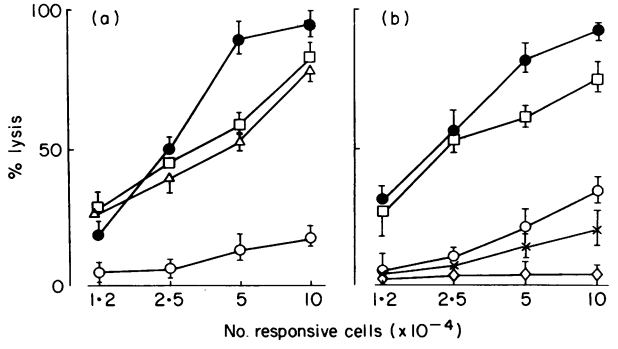

Figure 3. C57BL/6J BCG-spleen cells were stimulated with irradiated BDF_1 spleen cells in two different experiments. Percentage lysis was measured 4 days later; (○) control cultures; (□) supernatant from LPS-stimulated normal spleen cell cultures added; (△) LPS-containing tissue culture medium added; human IL-1 diluted to (×) 1 or (◊) 3 units per ml added; (●) response of normal spleen cells in medium (shown for comparison).

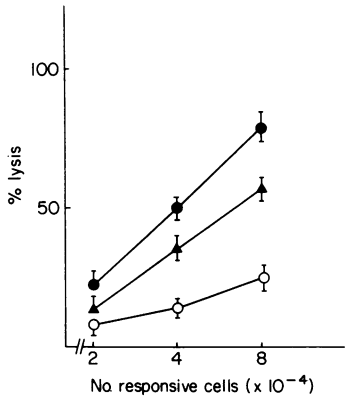

Figure 4. Normal spleen cells were stimulated with irradiated BDF_1 spleen cells either (●) alone or (○) in the presence of adherent BCG-spleen cells, or (▲) adherent BCG-spleen cells incubated overnight with LPS. Percentage lysis was measured 4 days later.

Effects of LPS and IL-1 on CMC responses by BCG-spleen cells

In order to study in more detail the effects of the IL-1-like factor(s) on the CMC responses of BCG-spleen cells, both crude LPS supernatants (in which the presence of IL-1-like activity had been shown in a separate assay) and purified human IL-1 were added to mixed cultures of C57BL/6J responder cells stimulated with BDF_1 spleen cells. The results shown in Fig. 3a demonstrate that the IL-1-containing supernatant stimulated the CMC reaction to a level comparable to that of the controls consisting of normal spleen cells. However, significant stimulation was also obtained with the addition to the cultures of LPS. Thus, the stimulation obtained in this experiment could have been due to a primary effect of LPS rather than to the IL-1-like activity contained in the supernatant. This conclusion is supported by the observation (Fig. 3b) that purified IL-1 not only did not enhance the response by the BCG-spleen cell population, but actually induced further suppression when used in the dose of 3 units per ml.

To gain some further insight into the mechanism of CMC amplification by LPS, adherent suppressor cells from the spleens of BCG-treated mice were incubated overnight with LPS and then washed and mixed with normal lymph node cells before adding BDF_1 stimulator cells for the MLC-CMC assay. The results shown in Fig. 4 demonstrate that the suppressor activity dropped significantly after incubation with LPS ($P < 0{\cdot}01$). This indicated that the adherent suppressor cells had been inactivated or destroyed by LPS. Data in support of the actual destruction of macrophage-like cells were obtained with the count of non-specific esterase-positive cells which dropped from 5–6% of total spleen cells from BCG-treated donors to less than 1% after incubation with LPS. On the other hand, no similar drop of esterase-positive cells was observed after incubation with Con A.

DISCUSSION

The initial objective of this investigation was to establish whether or not an interference with the production or the action of IL-1 may explain the profound suppression of CMC observed in BCG-treated C57BL/6J mice. IL-1 appears to be essential for T-cell activation, and provides not only a stimulus for proliferation but also for T-cell maturation (Mizel, 1982). The role of IL-1 in the *in vitro* generation of CTL is well established. IL-1 appears to induce the synthesis and secretion of IL-2 which, in turn, stimulates the proliferation of antigen-activated CTL precursors and their differentiation into fully active CTL (Smith, Gilbride & Favata, 1980; Farrar *et al.*, 1982). Since we had previously shown that the suppression of the *in vitro* generation of CTL by BCG was mostly due to a reduced production of IL-2-like factors (Kendall & Sabbadini, 1981), and since the data of the literature were not conclusive, it was logical to investigate a possible role of IL-1 in this reduced IL-2 production.

IL-1 activity was measured with the thymocyte assay which originally defined the biological activity of this monokine (Gery *et al.*, 1971). Since other lymphokines or monokines, especially IL-2, may also enhance thymocyte proliferation, it was considered necessary to complement the results of the thymocyte assay with an assay for IL-2 activity, which was negative in our experiments, and with gel filtration, which demonstrated the presence of proliferation-enhancing activity in fractions with molecular weights in the 14,000–20,000 range consistent with 15,000–17,000 MW. attributed to mouse IL-1 (Mizel, 1982). While these criteria do not prove in a definitive manner the IL-1 nature of the factor detected in the thymocyte assay, they justify referring to it as IL-1-like.

BCG induced a definite increase of IL-1-like activity, not only in the supernatants of LPS-stimulated cultures, but also in the supernatants of Con A- and alloantigen-stimulated cultures. LPS probably acts directly on macrophages and, therefore, may bypass

some of the physiological mechanisms that trigger IL-1 release. On the other hand, IL-1 release in Con A- and in alloantigen-stimulated cultures is probably secondary to an initial lymphocyte activation step (Mizel, 1982). Therefore, these results indicate that the initial recognition step was not suppressed, and that BCG suppression probably acts at a step of the reaction subsequent to the release of IL-1 and preceding IL-2 production. It would appear either that the interaction between IL-1 or similar factors and its target lymphocytes is interfered with, or that such targets are suppressed and cannot exert their helper function.

The lack of IL-1 involvement in the mechanism of suppression was confirmed by the similar increase of IL-1-like activity in C57BL/6J mice, which are suppressed by BCG and in CBA mice, the reactivity of which is potentiated by BCG, and was further supported by the failure to reverse BCG-induced suppression with the addition of purified human IL-1 to the cultures. The latter result is pertinent, in spite of the fact that exogenous IL-1 does not usually potentiate the CMC response of normal spleen cells (L. Mellow and E. Sabbadini, unpublished observations). If the suppression of BCG-spleen cells had been due to an IL-1 deficit, the exogenous addition of IL-1 would have been expected to restore a normal response. Since the human IL-1 preparation used in these experiments was fully active in the mouse thymocyte assay, it is unlikely that these negative results were due to the xenogeneic origin of the factor. The observation that high concentrations of IL-1 not only failed to reverse suppression, but actually increased it, may indicate that some suppressor cells are stimulated by IL-1. This argues against the possibility that suppressor molecules compete with IL-1, and supports the idea that the IL-1 targets are incapable of a normal response.

We have confirmed the presence of adherent cells capable of suppressing the *in vitro* generation of CTL and mitogenic reactions to Con A in spleens and lymph nodes from BCG-treated mice (Table 1). The BCG-induced adherent suppressor cells have been shown to be phagocytic (Klimpel & Henney, 1978) and, thus, to possess macrophage-like properties. Suppressor cells of this type are induced not only by BCG but also by the other bacteria (Scott, 1972; Bullock, Carlson & Gershon, 1978; Lichtenstein *et al.*, 1981a). Besides CTL responses, they suppress mitogen-induced responses (Schrier *et al.*, 1980; Turcotte, 1981) and antibody responses (Bullock *et al.*, 1978; Schrier *et al.*, 1980). The mechanism of action of these suppressor cells may consist of relating suppressor messages from suppressor T cells to other cells (Ptak, Zembala & Gershon, 1978), the production of suppressor factors (Nelson, 1973; Waldman & Gottlieb, 1973; Lichenstein *et al.*, 1981b), the modification of factors produced by lymphocytes (Tadakuma & Pierce, 1978), or may require direct cell-to-cell contact (Turcotte & Lemieux, 1982). Obviously, it cannot be excluded that T suppressor cells may also have been present in the lymphoid organs of our BCG-treated animals.

The addition of LPS to the cultures resulted in the abolition of the BCG-induced suppressor activity, probably through the inactivation or the destruction of macrophage-like suppressor cells. This explanation is supported by the observation that the removal of adherent cells resulted in a partial recovery of cytotoxic activity by BCG-suppressed cells (Table 1), and by the reduction of non-specific esterase-positive cells after incubation of BCG-spleen cells with LPS. Increased sensitivity of macrophages to LPS after BCG treatment has been described by others (Green *et al.*, 1976).

Suppressor factors were found in supernatants of the BCG-suppressed cultures. Some of these factors, sensitive to prostaglandin synthesis inhibitors (L. Mellow and E. Sabbadini, unpublished observations), were dialysable and, therefore, did not interfere with the present experiments which involved only dialysed supernatants. A higher molecular weight factor was also shown in MLC supernatants where it interfered with the detection of IL-1-like activity. This suppressor factor is antigen non-specific and does not impair cell viability (L. Mellow and E. Sabbadini, unpublished observations). Because of the presently insufficient characterization of this factor, it would be premature to make any comparisons with other suppressor factors described in the immunological literature. However, the detection of more than one suppressor activity indicates a great complexity of suppressor mechanisms triggered by BCG. This suggests the need for further studies about this factor. In the absence of more evidence, it is too soon to draw any conclusions about the role of this factor in suppression.

ACKNOWLEDGMENT

This work was supported by grants from the Medical Research Council of Canada.

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