INFECTION AND IMMUNITY, Mar. 1986, p. 826–831
0019-9567/86/030826-06$02.00/0

Vol. 51, No. 3

Mechanisms Underlying the Depressed Production of Interleukin-2 in Spleen and Lymph Node Cell Cultures of Mice Infected with *Mycobacterium bovis* BCG

R. TURCOTTE* AND D. LEGAULT

Bacteriology Research Center, Institut Armand-Frappier, Université du Quebec, Laval-des-Rapides, Ville de Laval, Québec, Canada, H7N 4Z3

Received 14 August 1985/Accepted 3 December 1985

Mice were infected intravenously with 1.0 mg of *Mycobacterium bovis* BCG. At various times thereafter, spleen and peripheral lymph node cells were stimulated with concanavalin A for 18 to 20 h, and their capacity to produce interleukin-2 (IL-2) was evaluated by means of a T-cell blast proliferation technique. A depression of IL-2 production that was complete in the spleen but partial in lymph node cell cultures occurred at 2 to 3 weeks and persisted till weeks 8 to 10 after infection. No direct evidence was found for an active suppressor mechanism depressing in vitro the production of IL-2. In spleen cell cultures the suppression of IL-2 production would result from a functional defect of the IL-2-producing T-cell subset, whereas in lymph node cell cultures the depression mainly results from a relative lack of IL-2-producing cells caused by an accumulation of immunoglobulin-positive and "null" cells. Spleen cells from BCG-infected mice maintained their capacity to acquire IL-2 receptors when activated by concanavalin A.

The antigen-nonspecific soluble factors, commonly called interleukins, appear to play a major role in the regulation of T cell-dependent immune responses, especially in the induction of T-cell proliferation and generation of effector cells (for review, see references 7 and 24). It is generally thought that the macrophage-derived interleukin-1 (IL-1) interacts with the mitogen- or antigen-primed helper T cells, leading to the production of interleukin-2 (IL-2). Once released, IL-2 binds to specific membrane-binding sites (IL-2 receptors) which are present on activated T cells, and thus the cells proliferate and differentiate into effector cells (18, 19).

Several in vivo and in vitro T-cell immune responses, such as the delayed-type hypersensitivity to tuberculin (4, 22), the antibody response to sheep erythrocytes (3), the lymphoproliferative responses to polyclonal mitogens (13), alloantigens (8), and specific antigens (5), and the generation of cytotoxic T-cell response (15) are severely impaired in mice infected with various species of mycobacteria or inoculated systemically with a massive dose of the BCG strain of *Mycobacterium bovis*. In addition, nonspecific suppressor cells (macrophagelike cells, T lymphocytes, or both) or inhibitory factors (or both) have been shown to be involved in the depression of most of these cellular responses (5, 8, 15, 21, 23).

Because of the major role played by IL-2 in T-cell proliferation and differentiation, a series of experiments was undertaken to investigate the relationship between the IL-2 activity and the proliferative response to T-cell mitogens in lymphoid cell cultures of mice infected systemically with a massive dose of BCG. In the present study, the kinetics of IL-2 production during the course of infection and the regulator mechanisms underlying IL-2 production were investigated in cultures of concanavalin A (ConA)-stimulated spleen and lymph node (LN) cells from infected mice.

Our results indicate a transient depression in the capacity of spleen and LN cells of infected mice to produce IL-2. The depression was more severe and sustained in the spleen cells than in the LN cell cultures and was closely related to the depressed proliferative response of these lymphoid cells to ConA. However, the mechanisms responsible for the depressed IL-2 production in the LN cells completely differed from those involved in spleen cell cultures.

MATERIALS AND METHODS

Infection of mice. Female C57BL/6 mice, obtained from the Canadian Breeding Farm & Labs Ltd., St. Constant, Quebec, were 2 to 3 months of age at the time of infection. Mice were injected intravenously with 1.0 mg (about 10^7 CFU) of a lyophilized preparation of the Montreal substrain of BCG (Institut Armand-Frappier) suspended in 0.1 ml of sterile saline. At 1-week intervals or, when specified, at 3 weeks after infection, single cell suspensions were prepared as described earlier (21), from the spleen and peripheral (popliteal, inguinal, and axillary) LNs of BCG-infected and of age-matched uninfected mice, as controls.

Culture medium and chemical reagents. RPMI 1640 (Flow Laboratories, Mississauga, Ontario), supplemented with 2 mM glutamine, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and the appropriate concentration of heat-inactivated fetal calf serum (GIBCO Canada, Burlington, Ontario) was used in the present experiments.

ConA and α-*methyl*-D-mannoside were purchased from Calbiochem (La Jolla, Calif.) and Sigma Chemical Co. (St. Louis, Mo.), respectively.

ConA proliferative assay. The mitogenic responses of spleen and LN cells to ConA were determined by measuring the incorporation of [^{3}H]thymidine into DNA as described previously (21). Briefly, viable lymphoid cells (5×10^5 per well in 96-well flat-bottomed tissue culture plate) were cultured for 3 days in 0.2 ml of culture medium supplemented with 10% fetal calf serum and 2.0 μg of ConA per well. On day 2 of culture, 1.0 μCi of [^{3}H]thymidine was added to each well, and the cultures were harvested 18 h later on filter pads with a Titertek cell harvester. Radioac-

* Corresponding author.

tivity was determined by scintillation counting, and the results were expressed as the mean counts per minute observed in triplicate cultures.

Assay for suppressive activity. Splenic suppressor cells were assayed for their capacity to inhibit the ConA-induced blastogenic response of normal splenocytes. Spleen cells from BCG-infected mice or from normal mice (hereafter referred to as BCG spleen cells and normal spleen cells, respectively) were cocultured in triplicate at three different ratios (1:2, 1:4, and 1:8) with normal splenocytes (4.0×10^6 per ml) as described above. The percentage of suppression was calculated at each cellular ratio according to the following equation: percentage of suppression = $1 - (cpm^{BCG}/cpm^{normal}) \times 100\%$, where cpm^{BCG} and cpm^{normal} represent the mean incorporation by the normal spleen cells in the presence of splenocytes, respectively, from BCG-infected and normal mice.

BCG spleen cells, at various concentrations (see below), were also assayed for their capacity to inhibit the production of IL-2 by normal spleen cells.

IL-2 production and titration. Normal and BCG spleen and LN cells were adjusted (unless otherwise stated) to 5×10^6 cells per ml of culture medium supplemented with 5.0 μM 2-mercaptoethanol, 5 μg of ConA per ml, and 5% fetal calf serum. After 18 to 20 h of incubation, the supernatants were harvested, supplemented with α-*methyl*-D-mannoside (20 mg/ml), filtered through 0.22-μm Acrodisc filters (Gelman Sciences, Inc. Ann Arbor, Mich.), and stored at −20°C until testing for IL-2 activity.

IL-2 activity was titrated by the ability to promote the growth of ConA-activated T-cell blasts which were prepared from normal mouse splenocytes, as described by Andersson et al. (1). T-cell blasts (10^4 per well in 96-well microculture plates) were incubated for 72 h with $\log_2$ serial dilutions of supernatants to be tested and then pulsed for 6 h with 1.0 μCi of [^{3}H]TdR per well. Each dilution of supernatants was tested in triplicate. Cells were harvested, and radioactivity was measured as stated above. In all titration experiments, a reference supernatant prepared from normal mouse splenocytes was titrated to determine maximum cpm and 50% of the maximum response (cpm 50% max). The dilution of tested supernatants giving cpm 50% max was found by using a method of probit analysis, as described by Gillis et al. (10). The IL-2 activity, expressed as units per milliliter of supernatant, was calculated as follows: IL-2 U/ml = (dilution cpm 50% max of tested supernatant)/(dilution cpm 50% max of reference supernatant).

Partial purification of IL-2. Supernatants from ConA-stimulated normal and BCG spleen cell cultures were first concentrated on a PM-10 Amicon membrane and then fractionated by preparative flat-bed isoelectric focusing in a pH gradient from 3.0 to 10.5. After dialysis against RPMI 1640 and lyophilization, each fraction was tested for IL-2 activity (see above) and for suppressive activity by evaluating their capacity to inhibit the ConA-induced blastogenic response of normal splenocytes as described before (23).

Evaluation of the spleen and LN cell subsets. The immunoglobulin-positive (Ig^+) and Ig^- cells were isolated from the spleen and LN cell populations by the panning technique of Wysocki and Sato (26). The Ig^- cells represent the nonadherent population of cells layered on plastic petri dishes coated with affinity-purified goat anti-mouse immunoglobulins (Kirkegaard & Perry Labs, Inc., Gaithersburg, Md.), whereas Ig^+ cells were recovered from the dishes coated with the same antibody preparation but diluted 1:10 in normal goat immunoglobulin G.

Cytotoxicity techniques were used for evaluating Thy-1.2^+, Lyt-1.2^+, and Lyt-2.2^+ cells in the whole spleen or LN cell suspensions. All biological reagents (monoclonal antibodies, Low-Tox rabbit complement, cytotoxicity medium) were purchased from Cedarlane Laboratories Ltd., Hornby, Ontario, and used under the experimental conditions recommended by these Laboratories. Data were expressed as percentage of the whole spleen or LN cells.

Absorption of IL-2 activity. Fresh and 48-h ConA-activated spleen cells from normal and infected mice were adjusted to 2×10^7 cells per ml of IL-2-containing supernatants and incubated for 1 h at 37°C.

Statistical analysis. Data are expressed as means ± standard errors of the mean. Student's *t* test was applied to the data.

RESULTS

Kinetics of IL-2 production in the spleen and LNs of BCG-infected mice. In noninfected control mice (time 0), the amount of IL-2 produced by the peripheral LN cells (1.11 ± 0.20 U) did not significantly differ from that produced by the spleen cells (1.03 ± 0.12 units, mean ± standard error of the mean from eight experiments) (Fig. 1). In infected mice, both the spleen and LN cells showed a marked depression in their capacity to produce IL-2. In spleen cell cultures, the depression was complete as early as 2 weeks postinfection, whereas in LN cells the IL-2 production reached its lowest value 1 week later. Then, near the 6 to 8 weeks or a few weeks later in other similar experiments, the lymphoid cells recovered gradually their capacity to produce IL-2. In all these experiments, the depressed capacity of spleen cells to produce IL-2 was more severe and sustained by comparison with LN cells.

Relationship between the capacity of spleen cells to produce IL-2 and to suppress the ConA-induced mitogenic response. The possible role of a suppressor mechanism in the depression of IL-2 production in spleen cell cultures was next investigated. For this study, spleen cells from BCG-infected mice were isolated at different times after infection and divided into two parts; one part of the cells was used to evaluate their capacity to produce IL-2, and the second part was used to determine their capacity to inhibit the ConA-induced proliferative response of normal splenocytes. The production of IL-2 was markedly depressed at 2 weeks after infection, that is, at the time when the suppressor activity

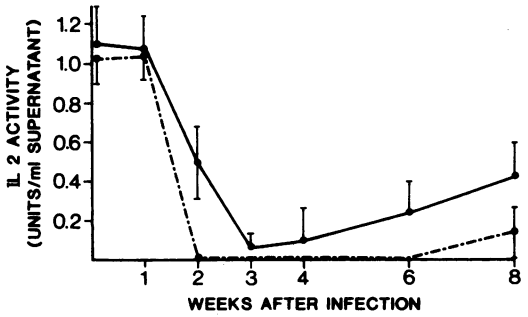

FIG. 1. Time course of IL-2 production in the spleens and peripheral LNs of BCG-infected mice. Each point represents the mean IL-2 activity ± the standard error of the mean in culture supernatants of spleen (-----) and LN (——) cell cultures obtained from three infected mice, except for uninfected mice (time 0), where the values were derived from 8 mice. The LNs and spleens originated from the same donor mice.

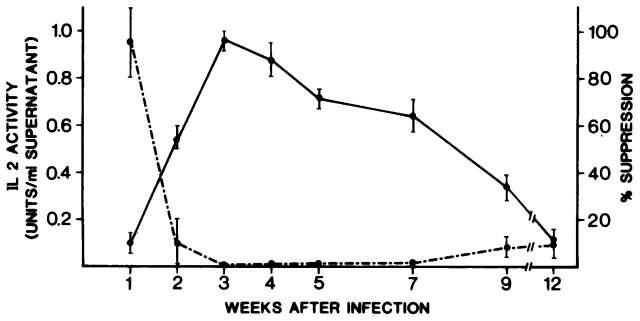

FIG. 2. Relationship between the capacity of spleen cells from BCG-infected mice to produce IL-2 and to suppress the ConA-induced proliferative response. Each point represents the mean IL-2 activity (-----) or suppressor activity (——) ± the standard error of the mean obtained from three mice. The spleen cells from each donor mouse served in both assays. The percentage of suppression obtained at a BCG/normal spleen cell ratio of 1:4 is presented.

reached significant levels (Fig. 2). A negligible amount or no amount of IL-2 was produced during the interval of high suppressive activity, that is, between weeks 3 and 7. Finally, a slight restoration of IL-2 production became apparent when the suppressor activity reached lower values. A close inverse relationship would thus exist between these two cellular responses.

Production of IL-2 from mixed spleen cell cultures. A more direct approach was used to investigate whether suppressor cells played an in vitro role in the depression of IL-2 production. Thus, a constant amount of normal spleen cells (5.0×10^6 per ml) were mixed with increasing amounts of BCG spleen cells just before ConA stimulation for the production of IL-2. The IL-2 activity detected in supernatants of mixed cultures decreased concomitantly with the increased number of BCG spleen cells added to the cultures (Table 1). Surprisingly, even at a normal to BCG/spleen cell ratio of 1:1, the amount of IL-2 produced remained relatively elevated (0.62 and 0.68 U in experiments 1 and 2, respectively), whereas at this cellular ratio the ConA-induced proliferative response of mixed cultures was completely suppressed (data not shown). The depressed IL-2 activity in mixed culture supernatants was not due to an exhaustion of essential nutrients in the culture medium, since within the range of cells used in the cultures, the IL-2 production by normal spleen cells increased constantly (Table 1). The partial depression of IL-2 activity observed in supernatants of mixed cultures could be due to suppressor cells interacting on IL-2-producing cells, to suppressor factors released into the culture supernatant by the BCG spleen cells during the 18 to 20 h of incubation and interfering with the detection of IL-2 by the T-cell blast technique, or to absorption by BCG spleen cells developing IL-2 receptors during the 20-h incubation period.

TABLE 1. Production of IL-2 in mixed spleen cell cultures

Composition of cultures[a]		U of IL-2 per ml of supernatant[b]	
Normal spleen cells (10^6)	BCG spleen cells (10^6)	Expt 1	Expt 2
5		1.00	1.00
5	0.62	0.92	0.89
5	1.25	0.86	0.83
5	2.5	0.74	0.75
5	5.0	0.62	0.68
	5.0	0	0

[a] Spleen cells from mice infected 3 weeks earlier with BCG were used in the mixed cultures.

[b] A value of 1.0 U was assigned to the unmixed control culture and served as the reference supernatant for calculating the number of units produced in the mixed cultures. The number of IL-2 units produced by normal spleen cells alone at densities of 5.62×10^6, 6.25×10^6, 7.5×10^6, and 10×10^6 cells per ml were 0.96, 1.17, 1.42, and 1.74, respectively, in experiment 1 and 1.07, 1.14, 1.57, and 1.71, respectively, in experiment 2.

TABLE 2. Inhibition of T-cell blast growth with culture supernatants prepared from ConA-stimulated spleen and LN cells of BCG-infected mice

Additions to T-cell blast culture	Vol/vol ratio	[^{3}H]thymidine incorporation[a] (cpm, 10^3)	% Suppression[b]
Fresh medium alone		0.2 ± 0.01	
Reference supernatant[c]:			
+ Fresh medium	1:1	34.7 ± 2.1	
+ Spleen cell supernatant	1:1	18.6 ± 0.9	46
	1:0.5	23.0 ± 1.2	31
	1:0.25	33.3 ± 1.8	4
+ LN cell supernatant	1:1	32.2 ± 2.1	7
	1:0.5	32.0 ± 1.9	8
	1:0.25	35.9 ± 2.4	0

[a] Mean incorporation ± standard error of the mean of triplicate cultures of T-cell blasts (5×10^4 cells per well).

[b] When compared with reference supernatant mixed 1:1 with fresh medium.

[c] IL-2-containing reference supernatant was prepared from ConA-activated normal spleen cells. It was mixed with supernatants from ConA-activated spleen and LN cells of infected mice at a volume/volume ratio as indicated. The twofold dilutions of spleen and LN cell supernatants were made in fresh culture medium before their addition to the reference supernatant.

Inhibition of T-cell blast growth with culture supernatants from infected mice. Soluble suppressor factors were searched in the BCG spleen and LN cell culture supernatants as follows. Serial twofold dilutions (in fresh culture medium) of supernatants of ConA-activated spleen or LN cell cultures from infected mice were mixed with the reference IL-2-containing supernatant, and the mixed supernatants were assayed for proliferative responses on T-cell blasts. The results of a typical experiment (Table 2) show that a significant inhibitory effect was present in the spleen cell supernatants and that this effect was dose related. In contrast, a very low or no suppressor activity was found in LN cell supernatants whatever the dilutions used. Thus, suppressor factors can interfere, at least in part, with the detection of IL-2 activity by the T-cell blast technique.

Partial purification of IL-2 and suppressor factors in culture supernatants of infected mice. To investigate the possibility that spleen cells from infected mice could produce IL-2 to a significant extent but that this activity is masked by the presence of suppressor factors, IL-2-rich supernatants and suppressor factor-containing supernatants obtained, respectively, from ConA-stimulated normal and BCG spleen cells were fractionated by preparative flat-bed isoelectric focusing, and each fraction was tested for IL-2 activity and suppressor activity (Fig. 3). With the normal spleen cell-derived supernatants, the IL-2 activity was detected in the pH 5.0 fraction, which was in good agreement with results obtained by previous workers using this technique (25). With supernatants from infected spleen cells, most of the suppressor activity was retained near the pH 3.0 to 4.5 fractions and near the pH 7.0 to 7.5 fractions, whereas negligible or no IL-2 activity was detected in the pH 5.0 fraction.

Depression in the capacity of LN cells to proliferate in

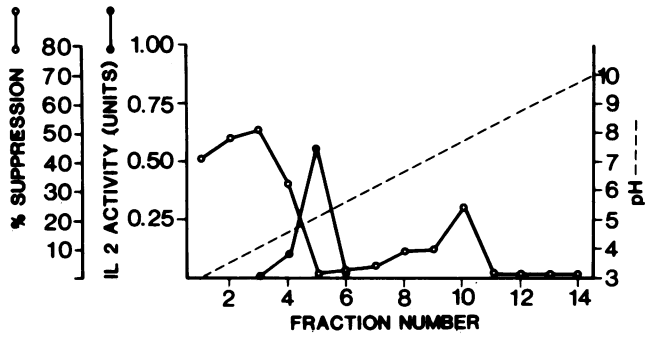

FIG. 3. Isoelectric focusing of ConA-stimulated spleen cell supernatants from normal and BCG-infected mice. The IL-2-containing fractions (●) were derived from the culture supernatants of normal mice. The IL-2 activity of fractions was evaluated by the T-cell blast technique by using the original nonfractionated preparation as the reference supernatant. The suppressor factor-containing fractions (○) were derived from culture supernatants of BCG-infected mice. The suppressor activity of fractions was evaluated by their ability to inhibit thymidine incorporation by normal spleen cells when mixed at 50% concentration with culture medium.

response to ConA. The proliferative response to ConA was markedly depressed in LN cell cultures of infected mice by comparison with the cellular response of normal mice ($P < 0.001$) (Table 3). Inversely, the proliferative response to lipopolysaccharide was significantly increased ($P < 0.02$). This situation, however, might be related to the high level of spontaneous incorporation detected in unstimulated BCG LN cell cultures. The depressed response to ConA in LN cell cultures could be due to a relative lack of ConA-responding cells rather than to a suppressor mechanism, since infected LN cells were unable to inhibit the mitogenic responses of normal spleen cells when used in mixed cultures (Table 3).

Effect of LN and spleen cell concentration upon the production of IL-2. To investigate the possibility that the depression of IL-2 production was due to a relative lack of IL-2-producing cells, increasing amounts of LN and spleen cells from mice infected 3 weeks earlier with BCG and from normal mice, as controls, were stimulated with a constant amount of ConA for the production of IL-2 (Table 4). With normal LN and spleen cells and, to a smaller extent, with infected LN cells, the production of IL-2 increased proportionally with the increased number of cells in the cultures. In contrast, with the infected spleen cells, the IL-2 production did not vary as a function of the cellular concentration used. In additional experiments not shown here, the depressed IL-2 production in infected spleen and LN cells was due neither to an inappropriate concentration of ConA in the cultures nor to a change in the kinetics of IL-2 production.

Relative proportion of B lymphocytes and T-cell subsets in LNs and spleens of infected mice. The analysis of the LN and spleen lymphocytes was carried out in a second approach to investigate whether the depressed IL-2 production was due to a lack of IL-2-producing cells (Table 5). By comparison with uninfected mice, the percentage of Ig^+ cells significantly increased ($P < 0.05$) in the LNs but highly decreased ($P < 0.001$) in the spleens of infected mice. Similarily, an inverse situation was found concerning the Ig^- cells. A significant reduction of Thy-1.2^+ ($P < 0.01$), of Lyt-1.2^+ ($P < 0.001$), and of Lyt-2.2^+ ($P < 0.001$) cells was observed in the infected LNs, whereas all of these cell populations did not vary to a significant extent in the spleen of infected mice. Last, the proportion of null cells markedly ($P < 0.001$) increased in the spleens and LNs of BCG-infected mice.

Ability of ConA-activated spleen cells to absorb preformed IL-2. Fresh and ConA-activated spleen cells were prepared from normal and infected mice and mixed with the reference supernatant in an attempt to remove the IL-2 activity contained therein (Table 6). Activated cells from both normal

TABLE 4. Effect of LN and spleen cell concentration upon the production of IL-2[a]

No. of cells in culture (10^6)	IL-2 production (U/ml of supernatant)			
	LN cells		Spleen cells	
	Normal	BCG	Normal	BCG
5	1.00	0.04	1.01	0
10	2.65	0.10	2.50	0
15	6.10	0.24	5.51	0
20	ND	0.81	17.65	0

[a] LN and spleen cells from either normal mice or from mice infected 3 weeks earlier were cultured at the concentration as indicated for 20 h with a constant amount (5 μg/ml) of ConA, and the culture supernatants were evaluated for the presence of IL-2 by the T-cell blast technique. ND, Not done.

TABLE 3. Proliferative responses to ConA and lipopolysaccharide of LN cells from normal and BCG-infected mice

Origin (and no.)[a] of cells	[^{3}H]thymidine incorporation[b] (cpm, 10^3)		
	Cells stimulated with:		Unstimulated cells
	ConA	Lipopolysaccharide[c]	
Normal LN cells (5.0)	205.8 ± 24.6	22.4 ± 9.7	3.3 ± 0.9
BCG LN cells[d] (5.0)	66.8 ± 5.6	43.3 ± 2.0	16.4 ± 1.9
Normal spleen cells (2.5)	107.5 ± 6.5	18.7 ± 5.7	1.5 ± 0.3
BCG LN cells (2.5)	51.4 ± 2.8	22.7 ± 3.0	9.4 ± 1.4
Normal spleen (2.5) + BCG LN cells (2.5)	122.4 ± 6.5	46.1 ± 4.7	18.2 ± 3.5

[a] Number of cells × 10^5 per well of tissue culture plate.
[b] Each data point represents the mean ± standard error of the mean of four separate experiments, one mouse per experiment, each performed in triplicate.
[c] Lipopolysaccharide was used at 50 μg/ml of culture medium.
[d] BCG LNs were removed from mice infected 3 weeks earlier.

TABLE 5. Percentage of T and B lymphocytes in the whole LN and spleen cell populations of normal and BCG-infected mice

Type of cells[a]	% of lymphocytes[b]					
	LN			Spleen		
	Normal	BCG[c]	P^d	Normal	BCG	P^d
Ig^+	35 ± 3	46 ± 2	<0.05	46 ± 3	24 ± 2	<0.001
Ig^-	44 ± 3	30 ± 3	<0.01	32 ± 2	44 ± 3	<0.01
Thy 1.2^+	55 ± 2	28 ± 3	<0.01	28 ± 2	31 ± 2	NS
Lyt 1.2^+	44 ± 2	19 ± 2	<0.001	27 ± 2	30 ± 3	NS
Lyt 2.2^+	30 ± 2	12 ± 1	<0.001	14 ± 1	15 ± 1	NS
Null cells	10 ± 2	26 ± 3	<0.001	26 ± 2	45 ± 3	<0.001

[a] Ig^+ and Ig^- cells were evaluated by a panning technique, whereas cytotoxicity techniques were used for evaluating Thy-1.2, Lyt-1.2, and Lyt-2.2 T lymphocytes (see Materials and Methods). The percentage of null cells was calculated from 100 − (%Ig^+ + %Thy-1.2^+).
[b] Each data point represents the mean ± standard error of the mean of at least four separate experiments, each performed in triplicate.
[c] LN and spleens were removed 3 weeks after infection.
[d] As determined by the Student t test. NS, Nonsignificant.

TABLE 6. Absorption of IL-2 activity with fresh and ConA-activated spleen cells from normal and BCG-infected mice

Absorption with[a]:	IL-2 activity (U/ml of supernatant)	
	Expt 1	Expt 2
None (control)	0.98	1.00
Normal spleen cells[b]:		
Fresh	1.03	1.03
ConA activated	0.17 (83)[c]	0.24 (77)
BCG spleen cells[b]:		
Fresh	1.57	1.21
ConA activated	0.43 (73)	0.44 (64)

[a] Portions of 1 ml of the reference supernatant (containing 1.0 U/ml) were incubated either alone (control) or with 2×10^7 fresh or 48-h ConA-activated spleen cells. After incubation at 37°C for 1 h, the cells were pelleted, and the IL-2 activity was determined in the supernatants by the T-cell blast technique.

[b] BCG and normal spleen cells came from mice infected 3 weeks earlier with BCG and from age-matched normal mice, respectively.

[c] Values within parentheses represent the percentages of absorption calculated from the value found in supernatants treated with corresponding fresh cells.

and infected mice had the capacity of absorbing IL-2 activity to a large extent, whereas the corresponding fresh cells were devoid of such a capacity. Activated BCG spleen cells might appear less efficient than activated normal cells in removing IL-2 activity. This situation seems to be related to the still-unexplained fact that more IL-2 was detected in the reference supernatant after treatment with fresh BCG spleen cells. However, when considering the percentage of absorption, activated BCG spleen cells were almost as efficient as activated normal cells in absorbing IL-2.

DISCUSSION

The kinetics of IL-2 production and the conditions controlling the production of IL-2 were examined in ConA-stimulated spleen and LN cell cultures of mice infected intravenously with BCG. The capacity of ConA-stimulated spleen cells to produce IL-2 was found to be abolished from week 2 or 3 until week 7 or 8 after infection. This finding might have some relevance with previous results which had shown a marked depression in the capacity of alloantigen-stimulated spleen cells from mice infected 3 weeks earlier with BCG to produce helper factors (14) and amplifying factors (16) of cytotoxic T-cell proliferation. The transient nature of the defect of IL-2 production, as found in the present study, is most likely related to the fact that BCG is nonpathogenic for the mouse. The production of IL-2 is presumably restored when the animal has completely recovered from the infection. In mice infected with the pathogenic *Mycobacterium lepraemurium*, the defect in IL-2 production by the spleen cells is of longer duration (12), even persisting until the death of the animal (R. Turcotte, unpublished results).

Some of the present data in combination with our previous data (21) reveal a good correlation between the capacity of lymphoid cells from BCG-infected mice to produce IL-2 and their ability to proliferate in response to ConA. Indeed, both of these cellular functions were abolished in spleen cell cultures, whereas they were partially depressed in LN cell cultures. Such an association between the lymphoblastic response and the production of IL-2 is therefore in good agreement with the well-established fact that T-cell proliferation is essentially dependent on IL-2 (7, 24).

The close relationship found, during the course of BCG infection, between the capacity of spleen cells to produce IL-2 and their ability to inhibit the lymphoproliferative response of normal splenocytes to ConA (Fig. 2) would suggest a suppressor mechanism regulating IL-2 production. This might be the case in vivo. However, this interpretation is not supported by the results obtained in mixed culture experiments in which infected spleen cells were unable to inhibit to a significant extent the IL-2 production by the normal spleen cells. Similar findings have also been reported recently in the murine malaria model (17). The partial depression of IL-2 activity detected in supernatants of the mixed BCG and normal splenocyte cultures (Table 1) was caused by the presence of contaminating suppressor factors as demonstrated by their ability to depress the proliferative response of the ConA-induced T-cell blasts (Table 2). Our previous results (23) and those of Colizzi et al. (6) had already shown that suppressor factors of mitogen-induced proliferation were spontaneously released into culture supernatants when either T lymphocytes or macrophages from the spleens of BCG-infected mice were incubated for 24 h. Together, these data suggest that the suppressor factors of ConA-induced T-cell blasts are produced by the infected spleen cells during the 20-h ConA stimulation for the production of IL-2 and that these factors are most likely identical to the nonspecific suppressor factors of DNA synthesis. On the other hand, the absence of a significant level of suppressor activity exerted by infected spleen cells on IL-2-secreting cells, as shown in this study, would contradict results reported recently by Colizzi et al. (6). These workers have succeeded in inhibiting the production of IL-2 by normal spleen cells by incubating them for 4 h with a BCG-induced T-derived suppressor factor before stimulation with ConA. Further experiments will be needed to investigate the possibility that under more appropriate experimental conditions, suppressor cells or factors (or both) might inhibit IL-2-producing cells.

Although suppressor factors of ConA-induced T-cell blasts were detected in the supernatants of BCG spleen cells, they cannot account for the lack of detection of IL-2 in these supernatants, since fractionation experiments designed to eliminate suppressor factors failed to reveal the presence of significant amounts of IL-2 in supernatants of BCG-infected spleen cells. Moreover, the lack of IL-2 production by the infected spleen cells would not result from a complete lack of IL-2-producing cells, since the percentage of Thy-1^+, Lyt-1^+, and Lyt-2^- cells which are mainly responsible for IL-2 production (24) did not significantly differ from the values found in the spleen of normal mice (Table 5). The production of IL-1, which is known to be essential for the stimulation of IL-2 generation (18, 19), was not depressed in cultures of macrophages isolated from mice infected either with BCG (R. Turcotte, unpublished results) or with other infectious agents (11, 12), thus implying that the depression of IL-2 generation by the BCG spleen cells was not due to a lack of IL-1. Finally, the fact that nonactivated BCG spleen cells did not bind IL-2 (Table 6) ruled out the possibility that IL-2 receptor cells, which could be induced in vivo by BCG, were already present in the cultures and were consuming any IL-2 produced during the 20-h incubation period.

The IL-2 receptor cell has been identified as a Thy-1^+, Lyt-1^-, Lyt-2^+ cytotoxic T-lymphocyte precursor (19). However, more recent evidence indicates that this T-cell subset could also be an IL-2-producing cell (2). Nevertheless, studies of absorption of IL-2 with ConA-activated spleen cells have revealed that IL-2 receptor cells can be

induced in spleen cell cultures or normal (20) and BCG-infected mice (Table 6). These data also suggest that the IL-2 receptor cells, like the IL-2-producing cells, would not be under the control of the BCG-induced suppressor cells of lymphoproliferation. On the other hand, the presence of these suppressor cells might explain why, in spite of several attempts, we have never succeeded in inducing T-cell blasts with spleen cells from BCG-infected mice.

A depressed capacity to produce IL-2, although less severe than in the spleen, also occurred in the peripheral LNs of mice infected systemically (the present study) and in the draining LN cells of those infected locally with BCG (R. Turcotte, unpublished results). A marked increase in the cellular content due to either recruitment, trapping, or proliferation in situ has been reported in the LNs of animals stimulated locally with various antigens and especially with bacterial adjuvants (27). Moreover, cells with B properties appear to accumulate to a much larger extent than T lymphocytes in stimulated LN cells (9). Similarly, after the intravenous administration of a large dose of BCG, a selective accumulation of cells with B properties and of null cells (Table 5) would occur in the peripheral LNs. This cellular accumulation consequently leads to a dilution of the IL-2 producing T cells. On the other hand, the possibility of an active suppressor mechanism of IL-2 production associated with a relative lack of IL-2-secreting cells cannot be completely excluded, since with infected LN cells, by opposition to normal LN and spleen cells, the production of IL-2 remained relatively low even when the number of infected LN cells was increased by a factor of 4 in the cultures (Table 4).

In conclusion, the present data strongly suggest that the depressed IL-2 production by the LN cells of BCG-infected mice resulted mainly from a relative lack of IL-2-producing cells. In contrast, the lack of production by the spleen cells of BCG-infected mice would result from a primary defect of IL-2 synthesis by the IL-2-producing cells rather than being secondary to an active suppressor mechanism.

ACKNOWLEDGMENTS

This work was supported in part by a grant (MA-8748) from the Medical Research Council of Canada.

We acknowledge the assistance of L. Richard in performing isoelectric focusing separations.

LITERATURE CITED

1. **Andersson, J., K. O. Gronvik, E. L. Larsson, and A. Coutinho.** 1979. Studies on T lymphocyte activation. I. Requirement for the mitogen dependent production of T cell growth factors. Eur. J. Immunol. **9**:581–587.
2. **Andrus, L., A. Granelli-Piperno, and E. Reich.** 1984. Cytotoxic T cells both produce and respond to interleukin 2. J. Exp. Med. **59**:647–652.
3. **Brown, C. A., I. N. Brown, and V. S. Sljivic.** 1979. Suppressed or enhanced antibody responses *in vitro* after BCG treatment of mice: Importance of BCG viability. Immunology **38**:481–488.
4. **Collins, F. M., and G. B. Mackaness.** 1970. The relationship of delayed hypersensitivity of acquired antituberculosis immunity. I. Tuberculin sensitivity and resistance to reinfection in BCG-vaccinated mice. Cell. Immunol. **1**:253–265.
5. **Collins, F. M., and S. R. Watson.** 1979. Suppressor T cells in BCG-infected mice. Infect. Immun. **25**:491–496.
6. **Colizzi, V., J. Ferluga, F. Garreau, M. Malkovsky, and G. L. Asherson.** 1984. Suppressor cells induced by BCG release non-specific factors *in vitro* which inhibit DNA synthesis and interleukin 2 production. Immunology **51**:65–71.
7. **Farrar, J. J., W. R. Benjamin, M. L. Hilfiker, M. Howard, W. L. Farrar, and J. Fuller-Farrar.** 1982. The biochemistry, biology and role of interleukin 2 in the induction of cytotoxic T cell and antibody-forming B cell responses. Immunol. Rev. **63**:129–166.
8. **Geffard, M., and S. Orbach-Arbouys.** 1976. Enhancement of T suppressor activity in mice by high doses of BCG. Cancer Immunol. Immunother. **1**:41–43.
9. **Gery, I., T. Navok, and Y. Stupp.** 1977. Selective accumulation of cells with B properties in stimulated lymph nodes. Immunology **33**:727–731.
10. **Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith.** 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. J. Immunol. **120**:2027–2032.
11. **Harel-Bellan, A., M. Joskowicz, D. Fradelizi, and H. Eisen.** 1983. Modification of T-cell proliferation and interleukin 2 production in mice infected with *Trypanosoma cruzi*. Proc. Natl. Acad. Sci. USA **80**:3466–3469.
12. **Hoffenbach, A., P. H. Lagrange, and M. A. Bach.** 1984. Influence of dose and route or *Mycobacterium lepraemurium* inoculation on the production of interleukin 1 and interleukin 2 in C57BL/6 mice. Infect. Immun. **44**:665–671.
13. **Huchet, R., and I. Florentin.** 1976. Studies on the mechanism of action of BCG, p. 85. *In* G. Lamoureux, R. Turcotte, and V. Portelance (ed.), BCG in cancer immunotherapy. Grune and Stratton, New York.
14. **Kendall, L., and E. Sabbadini.** 1981. Effect of Bacillus Calmette-Guérin on the *in vitro* generation of cytotoxic T lymphocytes. I. Effect of BCG on the frequency of cytotoxic T lymphocyte precursors and on the production of helper factors. J. Immunol. **127**:234–238.
15. **Klimpel, G. R., and C. S. Henney.** 1978. BCG-induced suppressor cells. Demonstration of a macrophage-like suppressor cell that inhibits cytotoxic T cell generation *in vitro*. J. Immunol. **120**:563–569.
16. **Klimpel, G. R., M. Okada, and C. S. Henney.** 1979. Inhibition of *in vitro* cytotoxic responses by BCG-induced macrophage-like suppressor cells. II. Suppression occurs at the level of a "helper" T cell. J. Immunol. **123**:350–357.
17. **Lelchuck, R., G. Rose, and J. H. L. Playfair.** 1984. Changes in the capacity of macrophages and T cells to produce interleukins during murine malaria infection. Cell. Immunol. **84**:253–263.
18. **Mizel, S. B.** 1982. Interleukin I and T cell activation. Immunol. Rev. **63**:51–72.
19. **Robb, R. J., A. Munck, and K. A. Smith.** 1981. T cell growth factor receptors. Quantification, specificity and biological relevance. J. Exp. Med. **154**:1455–1474.
20. **Stadler, B. M., S. F. Dougherty, J. J. Farrar, and J. J. Oppenheim.** 1981. Relationship of cell cycle to recovery of IL 2 activity from human mononuclear cells, human and mouse T cell lines. J. Immunol. **127**:1936–1940.
21. **Turcotte, R.** 1981. Evidence for two distinct populations of suppressor cells in the spleen of BCG-sensitized mice. Infect. Immun. **34**:315–322.
22. **Turcotte, R., and A. Forget.** 1983. Cutaneous unresponsiveness to *Mycobacterium bovis* BCG in intravenously infected mice. Infect. Immun. **41**:453–461.
23. **Turcotte, R., and S. Lemieux.** 1982. Mechanisms of action of *Mycobacterium bovis* BCG-induced suppressor cells in mitogen-induced blastogenesis. Infect. Immun. **36**:263–270.
24. **Watson, J. D., and D. Y. Mochisuki.** 1980. Interleukin 2: a class of T cell growth factors. Immunol. Rev. **51**:257–278.
25. **Watson, J. D., D. Y. Mochisuki, and S. Gillis.** 1983. Molecular characterization of interleukin 2. Fed. Proc. **42**:2747–2752.
26. **Wysocki, L. J., and V. L. Sato.** 1978. "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. USA **75**:2844–2848.
27. **Zatz, M.** 1976. Effects of BCG on lymphocyte trapping. J. Immunol. **116**:1587–1591.