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Unresponsiveness to Con A in spleen cell cultures of *M. lepraemurium*-infected mice is dependent on a defective expression of high-affinity IL-2 receptors rather than on a lack of IL-2 production

R. TURCOTTE* & S. LEMIEUX† *Applied Microbiology Research Center and †Immunology Research Center, Institut Armand-Frappier, Université du Québec, Laval-des-Rapides, Ville de Laval, Québec, Canada

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SUMMARY

The production of interleukin-2 (IL-2) by Con A-activated spleen cells (SC) progressively declined and reached negligible values during the course of infection of C57BL/6 mice with *Mycobacterium lepraemurium*. In addition, the capacity of cultured SC to utilize IL-2 was highly reduced, as demonstrated by the accumulation of IL-2 activity in culture supernatants at 48 and 72 h after Con A activation. The depressed IL-2 utilization started to be observed about 1 to 2 weeks prior to the onset of the depressed IL-2 production and was not reversed by the addition of exogenous IL-2; thus implying that a lack of IL-2 utilization rather than a lack of IL-2 production could be directly responsible for the inhibition of T-cell proliferative responses to Con A in SC cultures of infected mice. The utilization of IL-2 was found to be down-regulated, at least in part, by splenic suppressor cells since, in mixed-culture experiments, SC from infected mice actively depressed the capacity of normal splenocytes to consume IL-2. Finally, the depressed IL-2 utilization would result from a 2- to 3-fold reduction of either or both the density of high-affinity IL-2 receptors and their affinity for IL-2.

Keywords interleukin-2 high affinity receptors of IL-2 *M. lepraemurium* defective immune responses

INTRODUCTION

Lymphoid cells from susceptible mice, heavily infected with *Mycobacterium lepraemurium* have an impaired capacity to proliferate in response to mitogens, alloantigens and specific bacterial antigens (Navalkar, Patel & Kanchana, 1980; Turcotte, 1978; Yamaura, Akiyama & Nokano, 1981). Splenic suppressor cells, displaying a T-cell or a macrophage-like phenotype or both, are believed to be responsible, at least in part, for these defective immune responses (Ha, Lawton & Gardner, 1984; Hoffenbach, Lagrange & Bach, 1983b; Saito & Hirooka, 1983; Turcotte, 1978). It has also been shown that Con A-activated spleen cells from mice infected either with *M. lepraemurium* (Brett, 1984; Hoffenbach, Lagrange & Bach, 1983a) or with *M. bovis* BCG (Colizzi *et al.*, 1984; Turcotte & Legault, 1986) have also a reduced capacity to produce interleukin-2 (IL-2) whereas the production of interleukin-1 (IL-1) remains unaffected (Hoffenbach, Lagrange & Bach, 1984; Turcotte, unpublished results). These observations suggest that the reduced proliferative capacity of lymphocytes from mice infected with mycobacteria could be attributable to a lack of IL-2. However, in addition to a constant supply of IL-2, activated lymphocytes also need to express high-affinity IL-2 receptors (IL-2R) to be induced to proliferate (Larsson, 1981; Smith *et al.*, 1979). Even if IL-2 production and expression of IL-2R usually coincide and are both triggered by IL-1 (Haye *et al.*, 1984), they are likely to be related to distinct events in the multiple-step cascade driving to lymphocyte proliferation and differentiation (reviewed by Cantrel & Smith, 1984). Considering that Con A-activated blasts from *M. lepraemurium*-infected mice still show a reduced capacity to proliferate in the presence of exogenous IL-2, it has been hypothesized that a depressed capacity of activated lymphocytes to acquire IL-2R would also exist in these infected mice (Hoffenbach *et al.*, 1983a).

In the present study, we demonstrate that Con A-activated spleen cells from *M. lepraemurium*-infected mice have a reduced capacity to consume IL-2, a defect which was found to occur before the capacity to produce IL-2 was altered. The depressed IL-2 utilization would result from cellular suppressor mechanisms acting by reducing both the affinity and the density of high-affinity IL-2R on activated cells.

Correspondence: Dr R. Turcotte, Institut Armand-Frappier, 531 Blvd des Prairies, Laval-des-Rapides, Laval, Québec, Canada H7N 4Z3.

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 i.v. with 10^7 bacilli of the Hawaiian strain of *M. lepraemurium* freshly isolated from the liver and spleen of infected C57BL/6 mice, and suspended in 0·2 ml of PBS.

Production of interleukin-2

Spleen cells were adjusted to 5×10^6 cells per ml in RPMI-1640 medium supplemented with 5% heat-inactivated fetal calf serum (FCS), 2·0 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Flow Laboratories, Mississauga, Ontario), 50 μM 2-mercaptoethanol (J.T. Baker Chemical Co., Phillipsburg, NJ) and 5·0 μg/ml of Con A (Calbiochem, La Jolla, CA). Cells were seeded in 2-ml portions into wells of 24-well culture plates (Costar, Oxnard, CA) and maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. At 20, 48 and 72 h after the onset of incubation, cell culture supernatants were harvested by centrifugation ($500 \times g$ for 10 min), supplemented with α-methyl-D-mannoside (20 mg/ml, Sigma Chemical Co), filtered through 0·22 μm pore-size Acrodisc filters (Gelman Sciences, Inc., Ann Arbor, MI) and stored at minus 20°C until testing.

Assay for IL-2 activity

Culture supernatants were assayed for their ability to support proliferation of the IL-2-dependent mouse cytotoxic T-lymphocyte cell line (CTLL) according to a technique already described (Turcotte & Legault, 1986). In all titration experiments, performed in triplicate, a standard IL-2 preparation (Rat T-cell growth factor, Lot 86-1087 containing 2232 half maximal units (HMU) per vial, Collaborative Research, Inc., Lexington, Mass.) was titrated to determine maximum thymidine incorporation (ct/min) and 50% of the maximum ct/min. The IL-2 activity of tested supernatants was calculated by probit analysis (Gillis *et al.* 1978) and expressed in HMU per ml of culture supernatant.

Evaluation of splenic T-cell subpopulations

The relative proportions of Thy-1, 2^+, L3T4$^+$ and Ly-2$^+$ cells in spleen cell suspensions from normal and infected mice were determined by an indirect immunofluorescence staining technique. Unfractionated spleen cells (10^6/ml) were incubated for 45 min at 4°C with the appropriate rat monoclonal antibodies (Becton Dickinson Immunocytometry Systems, Mountain View, CA) under conditions recommended by the suppliers. The cells were washed twice and stained for 45 min at 4°C with a saturating concentration of an affinity purified, fluorescein-labelled goat anti-rat IgG (H+L) (Kirkergaard & Perry Labs Inc., Gaitherburg, MD) absorbed with mouse serum. After three washes, the percentage of cells with surface fluorescence was determined by flow cytometric analysis using an Epic-C Flow Cytometer (Coulter, Hialeah, FL). Background fluorescence, detected in cell preparations stained with the second reagent only was subtracted for calculations.

IL-2 binding assay

Spleen cells from normal and infected mice (alone or in admixture) were activated with Con A under conditions described above for the production of IL-2. The Con A-induced blasts were isolated by centrifugation ($500 \times g$ for 20 min at 20°C) over Ficoll-Hypaque, treated for 60 s with RPMI-1640 adjusted to pH 4·0 with 1 N HCl to remove receptor-bound IL-2 (Lowenthal *et al.*, 1986) and washed three times with RPMI-1640/FCS 5%. The binding of radiolabelled IL-2 (human recombinant [^{125}I]IL-2, New England Nuclear Research Products, Boston, MA) to the blast cells was performed according to the technique described by Robb, Munck & Smith (1981). The number of binding sites per blast and their dissociation constant (Kd) were derived by Scatchard analysis of equilibrium binding data using the 'Ligand' computer program developed by Munson & Rodbard (1980).

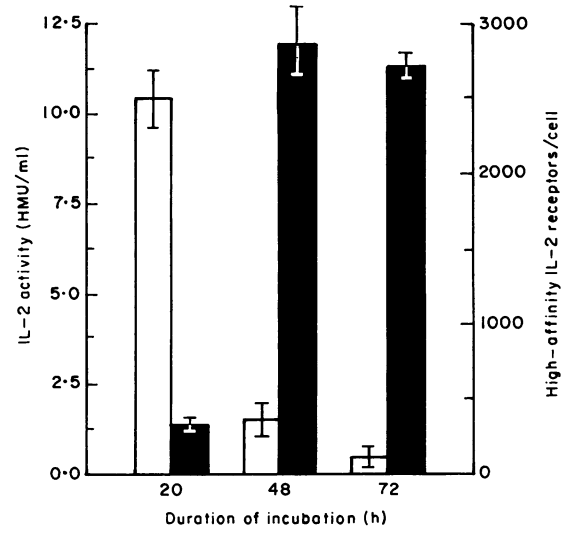

Fig. 1. Kinetics of IL-2 production by Con A-activated normal spleen cells and its utilization in relationship with the expression of high-affinity IL-2 receptors. Spleen cells (5×10^6/ml) were activated with 5 μg/ml of Con A. At 20, 48 and 72 h, culture supernatants were tested for IL-2 activity and the results (clear bars) expressed as HMU (half maximal units) per ml, whereas the number of high-affinity IL-2 R/blast cells (dark bars) was estimated by a radiolabelled IL-2 binding assay. Values are mean ± standard error of the mean of five independent experiments.

Lymphocyte proliferation assays

The proliferative responses of splenic T lymphocytes from normal and infected mice stimulated with Con A were determined by measuring the cellular incorporation of [^{3}H]TdR as described previously (Turcotte & Legault, 1986). In some experiments, the culture medium was supplemented with variable amounts of either IL-2-containing supernatants from murine cell cultures, or partially purified rat IL-2 (Collaborative Research, Inc., Lexington, MA), or human recombinant IL-2 (Boehringer Mannhein, Dorval, Quebec).

RESULTS

Kinetics of IL-2 production by Con A-activated normal spleen cells and its utilization in relationship with the expression of high-affinity IL-2 receptors

Spleen cells from normal C57BL/6 mice were incubated with Con A and after 20, 48 and 72 h of cultures the presence of IL-2 activity in culture supernatants was evaluated by the CTLL assay. At the same time, the density of high-affinity IL-2R on the blast cells was estimated by a radiolabelled IL-2 binding assay. Under these conditions, the production of IL-2 reached a maximum level after 18–20 h of incubation and then decreased

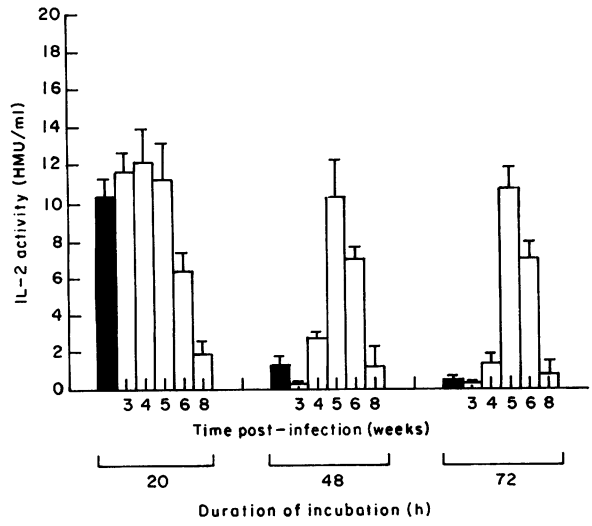

Fig. 2. Kinetics of IL-2 production in *M. lepraemurium*-infected mice at various times after infection. Mice were infected i.v. with 10^7 bacilli. At the indicated times post-infection, the spleen was removed and 2-ml aliquots containing 5×10^6 cells per ml were activated with Con A. IL-2 activity in culture supernatants was determined by the CTLL assay as in Fig. 1. Each column represents the mean IL-2 activity ± SEM obtained from at least four mice. Uninfected controls (■).

rapidly so that by 48 h of culture very little or no IL-2 activity was detected in culture supernatants (Fig. 1). The density of high-affinity IL-2R (Kd of 19–21 pM) on blast cells was low at 20 h of culture; then their number rapidly increased during the next 28 h and persisted at about the same level at 72 h (Fig. 1). Thus, an inverse relationship exists between the increase in the density of high-affinity IL-2R and the clearance of IL-2 from culture supernatants during the 20 to 48-h culture period. The Kd values of high-affinity IL-2R on Con A-activated blasts were 19·5 ± 0·9, 20·1 ± 0·9 and 21·4 ± 1·0 at 20, 48 and 72 h, respectively. This is in agreement with those reported by others on mitogen-activated murine splenocytes (Lowenthal *et al.*, 1986; Robb *et al.*, 1981).

Kinetics of IL-2 production and utilization by Con A-activated spleen cells from infected mice

The production of IL-2, evaluated in 20-h culture supernatants of Con A-activated SC harvested from mice infected with *M. lepraemurium*, was similar to that of normal SC up to 5 weeks post-infection; IL-2 production declined thereafter to reach very low values by week 8 (Fig. 2). From week 5 and until the end of the observation period, the level of IL-2 detected after 20 h of incubation either remained unchanged or slightly decreased in the 48- and 72-h culture supernatants. Thus, not only IL-2 production but also IL-2 utilization is impaired during the course of *M. lepraemurium* infection. As accumulation of IL-2 in the 48- and 72-h culture supernatants was detected at a time when IL-2 production was still normal (see week 5, Fig. 2), the defect in IL-2 utilization cannot be interpreted as just a consequence of insufficient IL-2 production.

Relative proportion of T-lymphocyte subsets in spleen of infected mice

The depressed capacity of SC from *M. lepraemurium*-infected mice to produce IL-2 and to consume IL-2 was not attributable to an intrinsic lack of the cell subsets required to assume these functions (Table 1). Indeed, until 8 weeks after infection, there was no significant perturbation in the number of $L3T4^+$ T cells which, upon Con A-activation, are the major producers of IL-2 and in the number of $L3T4^+$ and $Ly\text{-}2^+$ lymphocytes which, upon activation, will express IL-2 receptors (Malek, Schmidt & Shevach, 1985).

Table 1. Relative distribution of splenic T lymphocyte subsets in normal and *M. lepraemurium*-infected mice*

Weeks after infection	Percentage of cell expressing		
	Thy-1,2	L3T4	Ly-2
0	27·0 ± 1·8†	24·0 ± 1·3	15·7 ± 1·2
5	28·2 ± 1·1	24·2 ± 1·4	12·3 ± 1·3
8	25·5 ± 1·8	27·3 ± 0·3	13·8 ± 1·4

* Spleen cells from normal and infected mice were analyzed by an indirect immunofluorescence technique using a fluorocytometer (see Materials & Methods).

† Each data point represents the mean ± SEM of three separate experiments, each performed in duplicate.

Table 2. Affinity and number of IL-2 receptors in spleen cell cultures of *M. lepraemurium*-infected mice

Weeks after infection	Hours after cell activation	Affinity Kd (pM)*	No of binding sites/cell
0	20	19·5 ± 1·2†	320 ± 40
	48	19·2 ± 1·5	2641 ± 156
	72	19·7 ± 1·3	2901 ± 190
3	20	19·9 ± 3·5	451 ± 34
	48	22·9 ± 2·1	2488 ± 377
	72	21·1 ± 3·8	2654 ± 231
5	20	20·8 ± 1·2	490 ± 46
	48	37·1 ± 3·5	1024 ± 221
	72	36·4 ± 3·2	1282 ± 166

* The affinity of IL-2 receptors was estimated by an IL-2 binding assay under conditions defined in Materials & Methods' section.

† Each data point represents the mean ± SEM of three separate experiments, each performed with pooled spleen cells from 2 mice.

Density of high affinity IL-2 receptors in spleen cell cultures of infected mice

To investigate whether the depressed capacity of SC from *M. lepraemurium*-infected mice to consume IL-2, which was detected from 5 weeks post-infection (Fig. 2), was due to a lack of IL-2 binding sites on blast cells and/or to a depression of their affinity, SC from mice infected 3 and 5 weeks previously and from uninfected controls were activated with Con A and the

Table 3. Interrelationship between IL-2 production, IL-2 utilization and lymphocyte proliferation in Con A-stimulated cultures of spleen cells from normal and infected mice*

	Normal mice			Infected mice		
	IL-2 activity in supernatants at		[^{3}H]TdR incorporation at	IL-2 activity in supernatants at		[^{3}H]TdR incorporation at
Experiment no.	20 h	72 h	72 h	20 h	72 h	72 h
1	12·8†	2·1	169·3‡	13·9	10·9	104·8§
2	12·3	0·3	194·2	10·5	10·0	104·9§
3	10·5	0·5	152·7	8·3	8·0	34·8§
4	9·1	0·2	153·8	6·8§	4·8	86·9§
5	8·9	0·1	190·7	4·3§	3·6	48·8§
	$\bar{X}$ 10·7 ± 1·8	0·6 ± 0·8	172·1 ± 19·7			

* Mice infected 5 weeks earlier with 10^7 *M. lepraemurium* and aged-matched normal mice were used.

† HMU (half maximal units)/ml of culture supernatants.

‡ Mean counts per min ($\times 10^{-3}$) of triplicate cultures. The standard deviations being $\leq 10\%$ are not presented.

§ Statistically different ($P < 0.05$, at least) from the corresponding mean values of normal mice.

affinity and the density of IL-2 binding sites were determined at 20, 48 and 72 h after the onset of cultures. At 3 weeks post-infection, when SC from infected mice were still consuming IL-2 normally (Fig. 2), the Kd and the number of IL-2R per cell did not differ from those of uninfected mice (Table 2). On the other hand, in SC from mice infected 5 weeks previously, an approximately two-fold decrease in the affinity and in the density of IL-2R was observed not at 20 h but at 48 and 72 h of cultures. Additional experiments performed at 5 weeks post-infection revealed that the depressed affinity and density of IL-2R can occur either alone or in association.

Interrelationship between IL-2 production, IL-2 utilization and lymphocyte proliferation in Con A-stimulated cultures of spleen cells from infected mice

According to data reported in Fig. 2, the impairment of IL-2 utilization by Con A-stimulated SC, which occurs during the course of infection, takes place before the cells show a reduced capacity to produce IL-2. To establish if the *M. lepraemurium*-induced depressed capacity of splenic lymphocytes to proliferate is a consequence of impaired IL-2 production and/or utilization, these three lymphocyte properties were tested in Con A-stimulated cultures prepared with SC from five individual mice infected with *M. lepraemurium* 5 weeks before and compared with those of age-matched uninfected controls. As expected, in cell cultures in which a depressed capacity to produce IL-2 was observed, the proliferative capacity of the lymphocytes correlates with the quantity of IL-2 available (Table 3, Experiments 4 and 5). More importantly, a significant reduction of T-cell proliferation is detected as soon as the cells have a reduced capacity to consume IL-2 (Table 3, Experiments 1, 2 and 3).

In another series of experiments, different preparations of exogenous IL-2 (IL-2-containing supernatants from murine cell cultures, partially purified rat IL-2 and human recombinant IL-2) were added in increasing amount (up to 22 HMU/ml) at the initiation of cultures of SC from infected mice in an attempt to restore their blastogenic response to Con A. All three IL-2 preparations were found to stimulate, in a dose-dependent manner, [^{3}H]TdR uptake by the Con A-activated normal SC, but none of them reversed to a significant extent the depressed blastogenic response of SC from infected mice (data not shown), thus implying that the depressed Con A-induced proliferative response was not primarily due to a lack of IL-2.

Table 4. Effects of spleen cells from *M. lepraemurium* infected mice upon IL-2 production and utilization by normal splenocytes

Cells added to normal SC ($\times 10^{-6}$)*	IL-2 activity (HMU/ml) at 20 h	72 h
	Normal mouse	
0·62	5·8	0·1
1·25	7·8	0·2
2·50	9·8	0·4
	Infected mouse A	
0·62	5·6	4·8
1·25	3·1	2·8
2·50	1·2	1·3
	Infected mouse B	
0·62	6·5	0·2
1·25	4·0	2·0
2·50	1·3	1·5

* Spleen cells from two mice infected 8 weeks earlier and from one uninfected mouse were mixed with a constant number ($2{\cdot}5 \times 10^6$/ml) of normal splenocytes just prior to activation with Con A. At 20 and 72 h post activation, the IL-2 activity was determined in culture supernatants and expressed as half maximal units (HMU) per ml. $2{\cdot}5 \times 10^6$ normal SC alone gave rise to 5·2 HMU/ml.

Suppression of production and utilization of IL-2 by spleen cells from infected mice

Various numbers of SC from infected and normal age-matched mice, as controls, were co-cultured with a constant number ($2{\cdot}5 \times 10^6$/ml) of normal splenocytes in an attempt to inhibit the production and/or the utilization of IL-2 by the latter cells. The results of two representative experiments are shown in Table 4. As seen the presence of SC from infected mice interfered with the IL-2 production by normal splenocytes and this inhibitory effect was proportional to the dose of cells added to the cultures. Moreover, SC from infected mice inhibited IL-2 utilization but at different extent depending presumably on the immunodepressed status of the infected mice. Indeed, with SC from mouse A, an almost complete inhibition of IL-2 utilization was found since nearly all IL-2 activity produced earlier persisted in the 72 h-culture supernatants, whereas, in the second experiment, (mouse B) the inhibition of IL-2 consumption was observed only at high concentrations of added cells. These results would imply that suppressor mechanisms regulate the production and the consumption of IL-2 in *M. lepraemurium*-infected mice.

Density of high affinity IL-2 receptors in co-culture experiments

The mechanism by which SC from *M. lepraemurium*-infected mice depress IL-2 consumption by normal SC was investigated by determining the affinity and the number of IL-2 binding sites at 48 h post-activation on normal SC co-cultured with SC from infected mice. Spleen cells from individual mice infected 8 to 18 weeks previously were used at a 1 : 2 ratio with normal SC. Out of 10 infected mice investigated, three showed a depression in the affinity of IL-2R alone (Mean value of $52{\cdot}6 \pm 7{\cdot}7$ pM), three mice showed a depression in the number of IL-2 binding sites alone (1165 ± 54 per blast), whereas both defects were associated in the other four mice. Neither of these defects appeared to be related with the time course of the infection. Significant quantities of IL-2 were detected at 48 h in the supernatant derived from all these mixed cultures (unshown data), thus implying, as observed above, a defective IL-2 utilization by the blast cells. In other experiments not shown here, the extent of the depressed affinity and depressed number of IL-2 binding sites was found to correlate with the number of SC from infected mice added to the mixed cultures.

DISCUSSION

It is well established that in mitogen-activated murine or human lymphoid cell cultures, IL-2 detected in the supernatants peaks at 18–24 h after the initiation of the culture (Carlsson & Sjogren, 1985; Robb, 1984). It has also been shown, with quantitative IL-2 bioassays, that the following rapid decline in IL-2 activity is mainly attributable to an endocytosis process triggered by the binding of IL-2 to the high-affinity IL-2 receptors expressed on activated T-cells (Cantrel & Smith, 1984; Robb *et al.*, 1981). As shown in the present study, the density of high-affinity IL-2R (Kd of 19–21 pM) on the Con A-activated normal SC markedly increased between 20 and 48 h of culture; a situation, which would account, at least in part, for the rapid clearance of IL-2 activity from the culture supernatants (Fig. 1). Consequently, mitogen-induced T-cell proliferation would be affected if the number of IL-2 producing cells (mainly activated L3T4$^+$ cells) is reduced, if their triggering process is impaired or if their capacity to produce IL-2 is in any way inhibited by regulatory mechanisms. On the other hand, under conditions where IL-2 concentration is not critical, the main variables influencing T-cell proliferation will be the number of IL-2R-expressing cells as well as the density and the affinity of their surface receptors.

During the course of *M. lepraemurium* infection, IL-2 progressively accumulated in SC culture supernatants at 48 and 72 h after Con A stimulation (Fig. 2) instead of being utilized by activated lymphocytes. This accumulation was not due to a reduced proportion of L3T4$^+$ or Ly-2$^+$ T-cell subsets (Table 1) which upon activation are the cell populations mainly responsible for the removal of IL-2 from the supernatants (Malek *et al.*, 1985). On the other hand, the present study reveals a two-fold reduction in the Kd and in the density of high-affinity IL-2R in Con A-activated SC at 5 weeks after infection (Table 2). Even though no direct evidence is presented that such a relatively low reduction in the affinity of the IL-2R is responsible for the defect in IL-2 utilization, this interpretation would be in agreement with the fact that immature (Ly-2$^-$/L3T4$^-$) thymocytes, which express IL-2R with an affinity three- to five-fold lower than that of activated mature T-lymphocytes, are unable to endocytose bound IL-2 (Lowenthal *et al.*, 1986). The finding that reduced affinity and density of IL-2R were detected not at 20 h but at 48 and 72 h after Con A activation (Table 2) might also suggest that they are slowly developing culture-induced processes.

The production of IL-2 was itself markedly depressed in Con A-stimulated cultures of SC from *M. lepraemurium*-infected mice (Brett, 1984; Hoffenbach *et al.*, 1983a; and the present study). However, it appears unlikely that the depressed capacity of activated lymphocytes from infected mice to consume IL-2 might depend upon insufficient supply of IL-2 as the defect in IL-2 utilization was detected when normal levels of IL-2 were present in supernatants at 20 h (Fig. 2, week 5 and Table 3, Experiments 1, 2 and 3). Moreover, the addition of exogenous IL-2 failed to overcome the accumulation of IL-2 in supernatants after 48 and 72 h of cultures (data not shown). Such a depressed production of IL-2, associated with an impaired responsiveness to IL-2 and with a lack of restoration of the lymphoproliferative response by exogenous IL-2, has also been described in patients with active pulmonary tuberculosis (Toossi, Kleinhenz & Ellner, 1986), in lepromatous leprosy patients (Mohagheghpour *et al.*, 1985) and in mice infected with unrelated micro-organisms (Deschenes *et al.*, 1986; Harel-Bellan *et al.*, 1983; Reiner & Finke, 1983).

Cell mixing experiments demonstrate that the accumulation of IL-2 in SC cultures from *M. lepraemurium*-infected mice results from an active process mediated by suppressor cells (Table 4). In addition, these suppressor cells were found to exert their activity by down-regulating either or both the affinity and the density of IL-2R on activated cells. Consequently, at least two distinct mechanisms, acting independently and in association, would be involved in the depressed IL-2 utilization in SC cultures of *M. lepraemurium*-infected mice. Experiments are in progress to establish whether the depressed density of high-affinity IL-2R results from the shedding of these receptors from the cell surface, as demonstrated recently in cultures of human and mouse normal lymphoid cells (Osawa, Josimovic-Alasevic & Diamantstein, 1986; Rubin *et al.*, 1985) or from a defective mechanism in their synthesis or expression on the cell surface. Alternatively, competition for IL-2R by immunoregulatory molecules (e.g. suppressor factors) might also be involved in

reduction of their affinity. It has been shown that anti-receptor antibodies when complexed to IL-2R have the ability to depress the affinity of both high- and low-affinity IL-2 receptors (Lowenthal *et al.*, 1985).

The cell surface density of IL-2R on activated lymphocytes as well as their affinity for the ligand (Cantrel & Smith, 1984; Robb *et al.*, 1981) are critical variables for commitment to T-cell proliferation. Interestingly, the reduced capacity of Con A-activated SC from *M. lepraemurium*-infected mice to proliferate was detected as soon as the impaired responsiveness to IL-2 occurred (Table 3). Considering the kinetics of appearance of the observed defects, the impaired T-cell proliferation would primarily be the consequence of unresponsiveness to IL-2 rather than the outcome of a reduced capacity to produce IL-2. From these results, it is tempting to predict that, without preliminary measures aiming at correcting the defective IL-2 utilization, immunotherapy with IL-2 will be without any beneficial effect in acquired immunodeficiency diseases, such as those caused by Mycobacteria.

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