INFECTION AND IMMUNITY, Oct. 1981, p. 160-165
0019-9567/81/100160-06$02.00/0

Vol. 34, No. 1

Mechanisms in the In Vivo Release of Lymphokines: Relationship of High and Low Responsiveness to Other Parameters of the Immune Response

RUTH NETA[1] AND S. B. SALVIN[2]*

Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556[1], and Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261[2]

Received 22 January 1981/Accepted 12 June 1981

Variations exist between different strains of inbred mice in the release of lymphokines into the circulation. A number of manifestations of cell-mediated immunity in mice sensitized intravenously with *Mycobacterium bovis* BCG were analyzed to determine their association with the in vivo release of gamma interferon (IFN-γ) and migration inhibitory factor. Differences occurred among the strains in the proliferative responses of splenic cells to specific antigen and in the release of IFN after the challenge of BCG-sensitized mice with lipopolysaccharide. However, the capacity of an individual strain to release migration inhibitory factor and IFN-γ into the circulation did not parallel the extent either of the proliferative responses or of the release of IFN induced by lipopolysaccharide. Not all of the strains developed marked delayed footpad reactions to challenge with PPD regardless of the extent of their responses by other parameters. Delayed footpad reactions did develop in mice sensitized via the subcutaneous route, although this sensitization did not result in the capacity to release migration inhibitory factor and IFN-γ into the circulation of individual inbred strains.

The capacity of an animal to develop an immune response is under genetic control (12). The development of increased numbers of genetically defined strains of inbred mice provided an excellent means for the analysis of many of the basic mechanisms involved in the elicitation of the immune response. To identify the mechanisms involved in cell-mediated immunity (CMI), variations in resistance among different strains of mice have been studied (3, 10, 13, 17). Such investigations usually focused on differences between a resistant and a susceptible strain. Although such studies aid in an understanding of defects in the mechanisms of cellular resistance, an analysis of differences in the parameters of immune responses, after similar immunization of a large number of inbred strains, may identify the existence of different or alternate pathways leading to cellular immunity.

This immunity depends on the activity of T-lymphocytes and macrophages, which act and interact via the release of and response to lymphokines and monokines. Although a large number of lymphokines have been described (21), the exact role of particular lymphokines in resistance has not been determined. The possibility exists that the interaction of only a few specific lymphokines suffices for the development of resistance to a particular agent. In previous reports, inbred strains of mice were shown to release different types and quantities of six lymphokines after immunization with cell walls of *Mycobacterium bovis* strain BCG (15; R. Neta, S. B. Salvin, and M. Sabaawi, Cell. Immunol., in press). Of the 16 murine strains investigated for the capacity to release migration inhibitory factor (MIF) and gamma interferon (IFN-γ) into the circulation, 7 were low responders and 9 were high responders. The release of the four other lymphokine activities, i.e., that of mitogenic factor, of chemotactic factor, of cytotoxin, and of skin-reactive factor, did not correspond to this pattern. For example, DBA/1J mice, which do not release detectable quantities of MIF and IFN-γ into the circulation, do release chemotactic factor and skin-reactive factor (Neta et al., in press).

The questions therefore arise as to whether failure to release a given lymphokine is a selective defect or whether other manifestations of immune responses are similarly affected. Accordingly, experiments were initiated to examine the development of several other immune parameters in the various inbred strains of mice. In this paper, different strains of mice sensitized with cell walls of BCG were examined 3 weeks

later for (i) delayed and Arthus footpad reactions to purified protein derivative (PPD); (ii) proliferative responses of splenic cells to PPD, and (iii) release of IFN and MIF after challenge with bacterial lipopolysaccharide (LPS).

MATERIALS AND METHODS

Mice. Inbred strains of mice were purchased from Jackson Laboratories, Bar Harbor, Maine, namely, A/J, C57BL/6J, C57BL/10J, C57BL/10SN, BALB/cByJ, DBA/2J, C57BL/KsJ, Sec/1ReJ, AKR/J, CBA/CaJ, C3H/HeJ, RF/J, DBA/1J, and SJL/J, and the outbred female Swiss mice were purchased from Taconic Farms, Germantown, N. Y. Peritoneal-exudate cells were harvested about 5 days after intraperitoneal injection of light mineral oil.

Antigens. Cell walls from *M. bovis* strain BCG were prepared by and obtained through the courtesy of Edgar Ribi, Rocky Mountain Laboratory, Hamilton, Mont. The cell walls were ground in Drakeol and then emulsified in Tween 80-saline (CW/Dr) (19). In some experiments, mice were sensitized by intravenous injection of 300 μg of BCG cell walls in 0.2 ml. In other experiments, the mice were sensitized by subcutaneous injection (in the nape) of 300 μg of BCG cell walls in Freund (Difco Laboratories, Detroit, Mich.) adjuvant in a total volume of 0.2 ml. PPD, obtained either from Parke Davis & Co., Detroit, Mich., or from the National Institutes of Health, was used for footpad testing by injection of 30 μg in 0.05 ml in a hind footpad. A similar quantity of diluent was injected into the other hind footpad. Swelling of the footpad was measured with a Schnelltäster at 4 h postinjection for Arthus reactions and at 24 h postinjection for delayed-hypersensitivity reactions.

LPS was obtained through the courtesy of David Feingold, University of Pittsburgh School of Medicine. A 1- to 2-μg amount was injected intravenously into mice sensitized 3 weeks previously with CW/Dr.

MIF. This lymphokine was assayed by determining the dilution of experimental murine serum, the addition of which resulted in an inhibition of 20% or more of the migration of peritoneal-exudate cells on agar, in comparison with the effect of control serum from the same strain of inbred mice (18). A 0.6-ml amount of the serum dilution was mixed with 2.4 ml of an agar medium containing medium 199, 0.5% Ionagar (Oxoid), and 100 U of penicillin plus 100 μg of streptomycin per ml at 45°C, all dispensed in a volume of 3 ml in a 30-ml Falcon flask. A small drop of packed peritoneal-exudate cells was placed on the surface of the agar, and after incubation at 37°C for 48 h, the distance of migration was measured with the aid of a micrometer in a low-power microscope. The percentage of inhibition of migration was equal to

$$\frac{1 - \text{migration of peritoneal exudate cells in experimental serum}}{\text{migration of peritoneal exudate cells in control serum}} \times 100$$

The reciprocal of the maximum dilution of experimental serum causing a minimum of 20% inhibition was recorded as the titer of MIF activity.

Antigen-induced proliferation of splenic cells. The capacity of antigen to stimulate cell replication was measured with spleen cells from sensitized mice. The lymphoid cells were recovered from the interphase of a Ficoll-Hypaque gradient of teased splenic cells. A total of 4×10^5 of these cells in 0.1 ml were cultured in each well of a microtiter plate containing RPMI 1640 medium, with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer and 100 μg of PPD. After 48 h of incubation at 37°C in 5% CO_2, 1 μCi of tritiated thymidine was added to each well. Twenty-four hours later, the cells were harvested with a Mash II Systems harvester. The filter disks with the cellular material were put in scintillation vials with 9 ml of Econofluor, and the counts per minute of the samples were measured in a liquid scintillation counter.

IFN. IFN activity of a serum was determined by an assay involving the use of encephalomyocarditis virus hemagglutination reduction yield (8). L-929 cells were grown overnight in minimum essential medium (MEM) with 10% fetal calf serum in glass tissue culture tubes to form monolayers. The medium was decanted, and the cells were washed twice with phosphate-buffered saline. The control and experimental sera were diluted 10-fold to obtain 10^{-2} to 10^{-6} dilutions of each serum in MEM containing 2% fetal calf serum. The tubes with the cell monolayers were inoculated in triplicate with 1 ml of each of the serum dilutions. After a 24-h incubation at 37°C, the medium was removed and cell monolayers were washed with phosphate-buffered saline. 0.25 ml of encephalomyocarditis virus (hemagglutination titer, 1:16,384) in a buffer containing 0.5% gelatin and 0.2% lactoalbumin hydrolysate in Hanks solution was added to the monolayers. After 30 min of incubation at 37°C, the infected cells were washed twice with phosphate-buffered saline, MEM with 2% fetal calf serum was added, and the tubes were incubated for an additional 18 h. The virus was harvested by repeated freezing and thawing, and the titer was determined in a hemagglutination assay employing human "O" erythrocytes. The IFN titer was expressed as the reciprocal of the serum dilution that produced a reduction of 0.5 log hemagglutination yield of encephalomyocarditis virus. Ten units of IFN in this assay were equivalent to 1 U of a reference mouse IFN standard (obtained from the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases).

RESULTS

Comparison of Arthus and delayed-type hypersensitivities in inbred strains of mice. Inbred strains of mice sensitized intravenously with 300 μg of CW/Dr and challenged intravenously 3 weeks later released different quantities of IFN-γ and MIF into the circulation (15). In outbred Swiss-Webster mice, such sensitization and challenge induced the release of the two lymphokines into the circulation and enhanced specific CMI, but failed to stimulate marked delayed footpad hypersensitivity (19). It is not known whether this failure to develop delayed

footpad reactions is associated with the mode of antigen presentation or is determined by the genes controlling the specific immune response.

To investigate this problem, inbred strains of mice were immunized intravenously or subcutaneously with 300 μg of CW/Dr and 3 weeks later were tested in the footpads with 30 μg of PPD or with saline. The effect of strain variations and route of sensitization was measured by both delayed and Arthus reactivity (Table 1).

Footpad reactions after intravenous sensitization. The delayed footpad reactions of the intravenously sensitized mice were generally weak in all 14 strains, regardless of whether the strain was a high or low responder in the release of IFN-γ and MIF in vivo. Arthus reactions tended to vary from strain to strain. However, a lack of correlation with the levels of in vivo release of lymphokines was apparent. For example, high-responder strains in the release of IFN and MIF, such as DBA/2, had strong Arthus reactions, whereas others, such as C57BL/KsJ, had weak Arthus reactions. Low-responder strains in the release of the two lymphokines also varied in their Arthus responses, with mice of the BALB/cByJ strain having strong reactions and mice of the RF/J strain having weak reactions.

Footpad reactions after subcutaneous sensitization. Because marked delayed hypersensitivity did not develop in the various inbred strains after intravenous sensitization, this response cannot serve as an indicator of differences in CMI between strains. Since more intense footpad reactions developed after subcutaneous sensitization, this route was employed to compare Arthus and delayed reactions in the different strains (Table 1).

Subcutaneous sensitization of mice with CW/Dr, followed by footpad challenge with PPD, produced delayed and Arthus footpad reactions in all 11 strains tested. It should be noted that three of the strains that were low responders in the release of MIF and IFN-γ after intravenous sensitization (namely, AKR/J, CBA/CaJ, and C3H/HeJ) developed strong footpad reactions. However, all of the strains examined failed to release detectable quantities of MIF and IFN into the circulation after subcutaneous sensitization and intravenous challenge. Therefore, the expression of the two tested parameters of CMI, delayed footpad hypersensitivity and in vivo lymphokine release, in different strains depended primarily on the route of sensitization.

With the aid of the Spearman rank correlation coefficient, possible correlations between the following entities were examined: (i) Arthus reactions versus MIF release after intravenous sensitization; (ii) delayed footpad reactions versus MIF release after intravenous sensitization; (iii) delayed footpad reaction after intravenous versus subcutaneous sensitization; and (iv) Arthus reactions, after intravenous versus subcutaneous sensitization.

A correlation is not apparent in any of the four categories, with the possible exception of

TABLE 1. *Delayed and Arthus reactions to 30 μg of PPD after intravenous or subcutaneous sensitization with 300 μg CW/Dr*

Strain	Haplotype	Arthus reaction[a]		Delayed reaction		MIF after intravenous sensitization[b]	IF after intravenous sensitization[b]
		After intravenous sensitization	After subcutaneous sensitization	After intravenous sensitization	After subcutaneous sensitization		
A/J	a	−[c]	+	−	++	256	950,000
C57BL/6J	b	+	++	−	+	256	500,000
C57BL/10J	b	ND[d]	ND	+	+	256	1,300,000
C57BL/10SN	b	++	++	−	++	256	1,100,000
BALB/cByJ	b	++	+	+	++	0–32	<100–2,500
DBA/2J	b	++	+	+	++	256	120,000
C57BL/KsJ	b	+	+	−	++	256	1,300,000
Sec/1ReJ	b	−	ND	−	ND	64	200,000
AKR/J	k	+	++	+	++	4	1,100
CBA/CaJ	k	+	++	−	++	16	19,500
C3H/HeJ	k	++	++	−	++	4	850
RF/J	k	+	ND	−	ND	<4	<100
DBA/1J	q	+	+	+	+	<4	<100
SJL/J	s	−	ND	−	ND	64	260,000

[a] For correlation between parameters, see Table 2.

[b] Neta et al., Cell. Immunol., in press.

[c] Percent increase over control footpads. Each reading is derived from analyses of 4 to 12 mice. Symbols: −, <10% increase in thickness; +, 10 to 20% increase in thickness; ++, >20% increase in thickness.

[d] ND, Not done.

(ii), where a reverse correlation may exist (Table 2).

Proliferative responses to PPD of splenic cells from inbred murine strains. Proliferative responses of lymphoid cells to specific antigen have been used as an indicator of CMI. Therefore, proliferative responses of splenic cells from intravenously sensitized mice of different strains were measured to determine whether a correlation existed with other parameters of CMI, such as in vivo lymphokine release and delayed footpad response. Of the eight strains examined, not all of which developed footpad reactions to the specific antigen, three developed proliferative responses (Table 3). Of these three, two were high responders in the in vivo release of MIF and IFN-γ, i.e., C57BL/6J and C57BL/KsJ, and one, RF/J, was a low responder. In the other strains, the addition of PPD to sensitized splenic cells had either a suppressive effect or no discernible effect on cell proliferation. The suppressive effect was noted in both high-responder (e.g., A/J) and low-responder (e.g., CBA/CaJ) strains.

Effect of BCG-sensitization of different strains of inbred mice on release of IFN after challenge with LPS. Sensitization of mice with BCG, followed 3 weeks later by intravenous challenge with LPS, resulted in the release of increased quantities of IFN, in comparison with normal mice challenged with LPS (22). This enhanced release has been related to an increased activity of macrophages (9). If an increased production of IFN occurs in some sensitized mouse strains after challenge with LPS, in comparison with that occurring in nonsensitized mice, then activation of macrophages by BCG is suggested.

Of 10 strains studied, at least 5 released enhanced quantities of IFN. Of these five, two were low responders in the release of IFN-γ after old tuberculin challenge, namely, CBA/CaJ and DBA/1J (Table 4). Thus, the responsiveness of these two strains in the release of IFN after intravenous sensitization with BCG and intravenous challenge with LPS suggests that these

TABLE 2. *Correlation between some parameters of the immune response*

Parameter	Spearman rank correlation coefficient (r_s)
After intravenous sensitization	
Arthus reactions vs. MIF release	$r_s = -0.0865$ ($P = >0.05$)
Delayed reactions vs. MIF release	$r_s = -0.5606$ ($P = <0.05$)
Delayed footpad reactions	
After intravenous vs. subcutaneous sensitization	$r_s = 0.0432$ ($P = >0.05$)
Arthus reactions	
After intravenous vs. subcutaneous sensitization	$r_s = -0.1909$ ($P = >0.05$)

TABLE 4. *IFN and MIF titers in the sera of 10 strains of inbred mice sensitized intravenously with CW/Dr and challenged intravenously 3 weeks later with bacterial LPS or specific antigen old tuberculin (OT)*

Strain	IFN[a] titer after challenge with:		MIF titer after challenge with:	
	LPS[b]	OT	LPS[b]	OT
A/J	<100[c]	950,000[d]	<4	256
C57BL/6J	120	500,000	ND[e]	256
C57BL/10SN	17,000	1,100,000	16–32	256
BALB/cByJ	<100	<100–2,500	<4	<4–32
DBA/2J	1,100	120,000	ND	256
C57BL/KsJ	450	1,300,000	<4	256
AKR/J	200	1,100	<4	4
CBA/CaJ	1,150	19,500	<4	16
C3H/HeJ	<100	850	<4	4
DBA/1J	6,500	<100	<4	<4
SW (outbred)	11,000	1,000,000	ND	256

[a] Ten units equals 1 U of National Institutes of Health standard.
[b] Control mice challenged with 1 to 2 μg of LPS did not release detectable titers of IFN or MIF.
[c] Each reading represents a pool or pools of 4 to 12 mice.
[d] Each reading represents a pool or pools of 5 to 20 mice.
[e] ND, Not done.

TABLE 3. *Proliferative responses to 40 μg of PPD of splenic cells from eight inbred strains either normal or sensitized with 300 μg of CW/Dr*[a]

Strain	Sensitized[b] plus PPD	Sensitized	Δ cpm[c]	PPD	Normal	Δ cpm
A/J	3,301 (± 1,412)	6,722 (± 1,640)	Δ (−3,421)	3,518 (± 157)	4,142 (± 671)	Δ −624
C57BL/6J	9,441 (± 4,635)	3,820 (± 1,400)	Δ 5,621	4,792 (± 2,086)	2,065 (± 607)	Δ 2,907
C57BL/KsJ	4,753 (± 1,887)	1,639 (± 178)	Δ 3,114	1,746 (± 150)	887 (± 372)	Δ 859
Sec/1ReJ	4,049 (± 1,865)	4,600 (± 1,773)	Δ −551	5,413 (± 1,801)	3,551 (± 608)	Δ 1,862
AKR/J	794 (± 109)	712 (± 157)	Δ +82	1,044 (± 99)	1,790 (± 681)	Δ −746
CBA/CaJ	671 (± 97)	1,092 (± 280)	Δ −421	1,056 (± 101)	611 (± 128)	Δ +445
RF/J	31,594 (± 17,512)	6,260 (± 1,247)	Δ 25,334	16,335 (± 4,597)	7,150 (± 2,577)	Δ 9,185
SJL/J	572 (± 55)	1,097 (± 164)	Δ −525	757 (± 52)	1,571 (± 793)	Δ −814

[a] Results of one of two experiments. The second experiment produced results similar to these.
[b] Splenic cells harvested 3 weeks after intravenous sensitization with 300 μg of CW/Dr. Each figure is a mean of at least three spleens.
[c] Δ, Refers to difference in counts per minute in presence versus absence of PPD.

strains have the capacity to be activated by components of BCG.

DISCUSSION

Variations occur in the immune responses of different strains of mice to *Mycobacterium* sp. or its components. Thus, differences in susceptibility to infection were noted in 22 strains of mice challenged with *M. tuberculosis* H37Rv (16). Also, the capacity for marked granulomatous enlargement of the spleen and lung in response to intravenous injection of killed BCG was strain dependent, in that reactions did not occur in CBA/JCr and C3H/HeCr mice, but did occur in others, such as C57BL/6J and C57BL/KsJ (2). However, in nine inbred strains of mice treated with BCG, resistance to challenge with *Schistosoma mansoni* did not correlate with the degree of granulomatous response to BCG (4).

Similarly, the release of MIF and IFN-γ in vivo varied among inbred strains of mice (15; Neta et al., in press), but did not correlate with the capacity of splenic cells to proliferate to specific antigen (Table 3) or to concanavalin A, or with the capacity of a sensitized strain to release IFN in vivo in response to challenge with LPS (Table 4). Marked delayed footpad responses to PPD did not develop after intravenous sensitization in any of the 14 strains, although some strains did develop Arthus reactions (Table 1). The presence in some murine strains of some manifestations of CMI, in the absence of delayed footpad responses, may indicate that cells, presumably T-lymphocytes, capable of inducing delayed reactions, are present, but do not appear at the reactive site because of suppression or sequestration (11). Suppressor cells of the adherent (1, 6, 14, 20) or nonadherent (5, 7, 14) type have been observed in the spleens of mice sensitized with BCG. The presence of a suppressor cell has been associated with the capacity of a given strain to respond to an intravenous injection of killed BCG (1), i.e., the C57BL/6 strain that had marked granulomatous responses also had suppressor cells; the CBA strain that did not produce marked granulomas did not have suppressor cells. It appears that the suppressor cells develop in the process of granuloma formation. In contrast, the presence of a suppressor substance to IFN action has been associated with an apparent low responsiveness of mice to sensitization with BCG (R. Neta, Cell. Immunol., in press). Therefore, the apparent high or low responsiveness, as measured by a certain parameter, is a result of multiple, sometimes negating, interactions.

Results from the present experiments suggest that the various manifestations of CMI may be under separate control. Thus a strain, such as RF/J, that was a low responder in the in vivo release of MIF and IFN-γ (Table 1) had marked proliferative responses of splenic cells to PPD (Table 3), whereas a strain, such as A/J, which was a high responder in the in vivo release of MIF and IFN-γ (Table 1), showed inhibition of the proliferation of spleen cells in the presence of PPD (Table 3) and did not release enhanced quantitites of IFN upon challenge with LPS (Table 4). Strains C57BL/10SnJ and DBA/1J, which differed markedly in the quantities of MIF and IFN-γ released into the circulation after old tuberculin challenge (Table 1) both had high titers of IFN after challenge with LPS (Table 4).

The expression of parameters of CMI after sensitization does not depend solely on the genetic makeup, but is also determined by the manner of antigen presentation. Thus, subcutaneous administration of CW/Dr resulted in the absence of detectable titers of MIF and IFN-γ in the circulation of all the strains tested after old tuberculin challenge and in the development of marked delayed footpad reactions to PPD (Table 1). Strains, such as AKR/J, CBA/J, and C3H/HeJ, which were low responders in production of IFN-γ and MIF after intravenous sensitization (15) had the most pronounced footpad reactions to PPD after subcutaneous sensitization (Table 1).

In view of the dissociation of the different manifestations of CMI, the question arises as to what parameters are necessary for enhanced resistance to a particular agent. Studies correlating the parameters of CMI in different inbred strains with the development of resistance should be of aid in determining what responses or interactions are essential for the development of specific resistance.

ACKNOWLEDGMENTS

This work was supported by grants AI-16064 and AM-27727 from the Public Health Service, National Institutes of Health to S.B.S., by the American Lung Association, and by U.S. Public Health Service grant IF-32CA-06519 to R.N. from the National Institutes of Health.

The authors express their deep appreciation to Floyd H. Taylor for his expert advice and assistance in the statistical analysis of the data and to Robert B. Propes for his outstanding technical assistance.

LITERATURE CITED

1. **Allen, E. M., and V. L. Moore.** 1979. Suppression of phytohemagglutinizing and lipopolysaccharide responses in mouse spleen cells by Bacillus-Calmette-Guerin. RES J. Reticuloendothel. Soc. **26**:349–356.
2. **Allen, E. M., V. L. Moore, and J. O. Stevens.** 1977. Strain variation in BCG-induced chronic pulmonary inflammation in mice. I. Basic model and possible genetic control by non-H-2 genes. J. Immunol. **119**:343–

347.

3. **Cheers, C., and I. F. C. McKenzie.** 1978. Resistance and susceptibility of mice to bacterial infection: Genetics of listeriosis. Infect. Immun. **19**:763–770.
4. **Civil, R. H., and A. A. F. Mahmoud.** 1978. Genetic differences in BCG-induced resistance to *Schistsooma mansoni* are not controlled by genes within the major histocompatibility complex of the mouse. J. Immunol. **120**:1070–1072.
5. **Collins, F. M., and S. R. Watson.** 1979. Suppressor T-cells in BCG-infected mice. Infect. Immun. **25**:491–496.
6. **Florentin, I., R. Huchet, M. Bruley-Rosset, O. Halle-Pannenko, and G. Mathe.** 1976. Studies on the mechanisms of action of BCG. Cancer Immunol. Immunother. **1**:31–39.
7. **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.
8. **Jameson, P., M. A. Dixon, and S. E. Grossberg.** 1977. A sensitive interferon assay for many species of cells: encephalomyocarditis virus hemagglutinin yield reduction. Proc. Soc. Exp. Biol. Med. **155**:173–178.
9. **Lackovic, V., and L. Borecky.** 1974. Peritoneal leukocytes—central role in interferon production. IRCS **2**: 1080–1088.
10. **LaGrange, P. H., and B. Hurtrel.** 1979. Local immune response to *Mycobacterium lepraemurium* in C3H and C57Bl/6 mice. Clin. Exp. Immunol. **38**:461–474.
11. **LaGrange, P. H., and G. B. Mackaness.** 1978. Site of action of serum factors that block delayed type hypersensitivity in mice. J. Exp. Med. **148**:235–245.
12. **McDevitt, H. O., and B. Benacerraf.** 1969. Genetic control of specific immune responses. Adv. Immunol. **11**:31–74.
13. **Nakamura, M. R., and T. Tokunaga.** 1978. Strain differences of delayed-type hypersensitivity to BCG and its genetic control in mice. Infect. Immun. **22**:657–664.

13a. **Neta, R.** 1981. Mechanisms in the *in vivo* release of lymphokines. II. Regulation of in vivo release of type 2 interferon (IFN-γ). Cell. Immunol. **60**:100–108.

14. **Neta, R., and S. B. Salvin.** 1979. Adjuvants in the induction of suppressor cells. Infect. Immun. **23**:360–365.
15. **Neta, R., and S. B. Salvin.** 1980. *In vivo* release of lymphokines in different strains of mice. Cell. Immunol. **51**:173–178.
16. **Pierce, C., R. J. Dubos, and G. Middlebrook.** 1947. Injection of mice with mammalian tubercle bacilli grown in Tween-albumin liquid medium. J. Exp. Med. **36**:159–174.
17. **Plant, J., and A. A. Glynn.** 1976. Genetics of resistance to infection with *Salmonella typhimurium* in mice. J. Infect. Dis. **133**:72–78.
18. **Salvin, S. B., and J. Nishio.** 1969. *In vitro* cell reactions in delayed hypersensitivity. J. Immunol. **103**:138–141.
19. **Salvin, S. B., E. Ribi, D. L. Granger, and J. S. Youngner.** 1975. Migration inhibitory factor and type II interferon in the circulation of mice sensitized with mycobacterial components. J. Immunol. **114**:354–359.
20. **Turcotte, R., L. Lafleur, and M. Labreche.** 1978. Opposite effects of BCG on spleen and lymph node cells: lymphocyte proliferation and immunoglobulin synthesis. Infect. Immun. **21**:696–704.
21. **Waksman, B. H.** 1979. Modulation of immunity by soluble mediators. J. Pharmacol. Exp. Ther. **2**:623–672.
22. **Youngner, J. S., and W. R. Stinebring.** 1965. Interferon appearance stimulated by endotoxin, bacteria or viruses in mice pretreated with *E. coli* endotoxin or infected with *Mycobacterium tuberculosis*. Nature (London) **208**:456–458.