Suppressor T Cells for Delayed-type Hypersensitivity in Susceptible Mice Infected with *Mycobacterium lepraemurium*[1]

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Previous works from this ([14, 15]) and other laboratories ([2]) have demonstrated the presence of suppressor cells (macrophage-like and T lymphocytes) in the spleen of mice infected with *Mycobacterium lepraemurium* (*Mlm*). These suppressor cells are not antigenically specific since they can inhibit the lymphoproliferative response to polyclonal mitogens ([14, 15]), to alloantigens ([4]), and to specific antigens ([1]). In addition, they depressed several other *in vitro* immune functions, such as the antibody response to sheep erythrocytes ([2]), the generation of cytotoxic T-cell response, and the expression of natural-killing activity (Lemieux, *et al.*, unpublished results). So far, no well-defined role has been ascribed to these cells in the *in vivo* regulation of anti-*Mlm* immune responses, apart from the fact that the loss of the delayed-type hypersensitivity (DTH) reaction to mycobacterial antigens occurring in leprous mice appears to correspond to the time of the appearance of suppressor cells in their spleen ([2]). A series of experiments was, therefore, designed to study the role suppressor cells exert *in vivo,* especially on the development of the DTH response to *Mlm* antigens and the resistance of mice to *Mlm* infection.

In the present study, we have found that the T-cell-enriched population from the spleen of *Mlm*-infected mice, when adoptively transferred to syngeneic recipients, markedly depressed the induction and the expression of DTH to *Mlm* antigens. In addition, each of these biological manifestations seemed to be regulated by distinct subsets of T-suppressor cells.

MATERIALS AND METHODS

Mice. Female mice of the inbred strain C3H/HeNCrl, a strain highly susceptible to *M. lepraemurium* when infected subcutaneously ([15]), were purchased from Charles River Breeding Labs, Inc., Wilmington, Massachusetts, U.S.A. They weighed 16 to 18 g at the beginning of the experiments, and were maintained under standard laboratory conditions and fed Purina Chow and water *ad libitum.*

M. lepraemurium. The Hawaiian strain of *Mlm* was propagated in C3H mice as previously described ([14]). At the time of infection, fresh bacilli were isolated from the liver or spleen of infected mice and counted according to the slide technique of Shepard and McRae ([13]).

Immunodepression of mice. Mice were immunodepressed by an intravenous (i.v.) inoculation of 1×10^7 *Mlm* bacilli. Four months after infection, the mice were totally anergic to *Mlm* antigens (see below), and suppressor cells were detected in their spleen, as demonstrated by the suppression of the mitogen-induced lymphoproliferation when co-cultured at a 1:1 ratio with spleen cells from normal mice ([14]).

Separation of spleen cells. The spleens from immunodepressed and from age-matched control mice were removed under sterile conditions, and the lymphoid cells were isolated according to a method already described ([14]). Spleen cells (5×10^7 suspended in 30 ml of Hanks' balanced salt solution [HBSS] supplemented with 10% fetal calf serum) were first separated into adherent and nonadherent populations by a 2-hr incubation at 37°C in 150-mm sterile plastic Petri dishes coated with teflon ([5]).

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After flushing the dishes with HBSS, the adherent cells were harvested by scraping the bottom of the dishes with a rubber policeman. The nonadherent cells were further separated into Ig+ and Ig− cells according to the "panning" technique of Wysocki and Sato ([18]). The Ig− cells, hereafter referred to as enriched T 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, Maryland, U.S.A.), while Ig+ (B lymphocytes) were recovered from the dishes coated with the same antibody preparation but diluted 1:10 in normal goat IgG. An aliquot of the enriched T cells at a concentration of 1×10^7/ml of RPMI medium was irradiated with 2500 rads of gamma rays from a gamma cell 220 ^{60}Co irradiation unit (Atomic Energy of Canada, Ltd). In some experiments, the Lyt 1+ and Lyt 2+ lymphocytes were eliminated from the T-cell-enriched population by treatment with an appropriate monoclonal antibody and complement prior to adoptive transfers. All biological reagents (monoclonal anti-Lyt 1.1, anti-Lyt 2.1, Low-Tox rabbit complement, cytotoxicity medium) were purchased from Cedarlane Labs, Ltd, Hornby, Ontario, Canada, and used under the experimental conditions as recommended by these laboratories.

Prior to adoptive transfers, each cellular preparation was washed three times in HBSS, counted in a hemacytometer, and adjusted to the desired concentration in this medium.

Adoptive transfer of suppression. One ml of unseparated or separated spleen cells from immunodepressed (and noninfected, as controls) mice containing 1×10^7 cells was slowly injected via the tail vein into groups of either syngeneic normal mice or mice infected 5 weeks previously in one hindfoot pad with 1×10^7 *Mlm*. The groups of normal recipient mice were immediately infected in one hindfoot pad with 1×10^7 *Mlm* and challenged 5 weeks later in the opposite foot pad with *Mlm* antigens to study the effect of cell transfers on the induction of the DTH reaction. The infected groups of recipient mice were challenged 1 day after cell transfers with *Mlm* antigens to study the effect of adoptive cell transfers on the expression of the DTH reaction. All spleen-cell preparations from immunodepressed mice were contaminated with a substantial number of acid-fast bacilli (AFB). Histological analyses revealed that the bacilli adhered to the cell surface in the T- and B-lymphocyte preparations, whereas most of the bacilli resided in clumps and intracellularly in the adherent-cell preparation. Approximately 1.5×10^8 AFB were detected per 1×10^7 cells of the T-cell-enriched population (that is, per one transferred dose). To study the effects of contaminating bacilli on the induction and the expression of DTH, an additional group of mice (hereafter referred to as infected controls) was inoculated i.v. with 1.5×10^8 cell-free *Mlm* bacilli instead of the spleen-cell preparations at the time of adoptive transfers.

Preparation of skin-test antigens. A suspension containing 5×10^9 *Mlm* bacilli per ml of HBSS was sonicated for 20 min under cooling on ice using a Braunsonic 1510 sonicator (B. Braun Melsungen AG) set at 80 watts. The whole suspension was used as such to elicit DTH reactions.

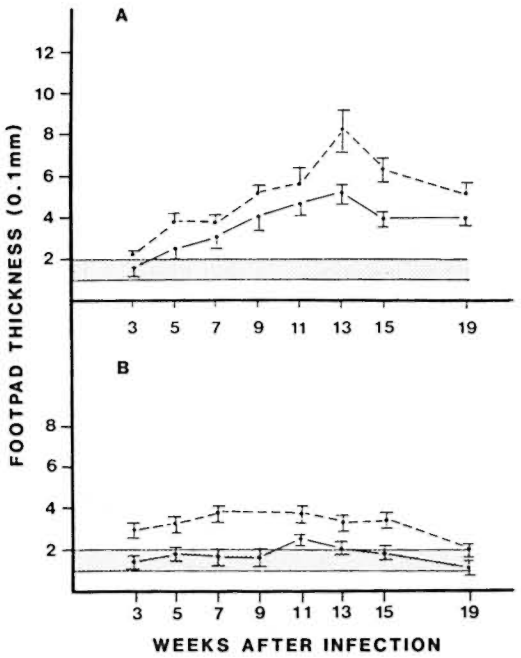
Delayed-type hypersensitivity reaction. DTH was evaluated by the foot pad swelling test. Mice were challenged with 20 μl of the whole sonicated preparation (equivalent to 10^8 bacilli) given into one hindfoot pad as recommended by Poulter and Lefford ([12]). The foot pad thickness was measured with dial-gauge calipers prior to the challenge and at 24 hr and 48 hr later, and expressed in 0.1 mm units. To study the specificity of *Mlm*-induced suppressor cells on the DTH reaction, groups of mice were sensitized in one hindfoot pad with 1×10^8 sheep red blood cells (SRBC) either 5 to 7 days prior to or immediately after the adoptive transfer of spleen cells from *Mlm*-immunodepressed mice. The animals were challenged in the contralateral foot pad with 1×10^8 SRBC, and the reaction was read 24 hr later as above.

Statistical analysis. All transfer experiments were repeated at least once. Each experimental group was composed of 5 to 7 mice. Results are expressed as the mean increase in foot pad thickness (in 0.1 mm) ± standard error of the mean (SEM). Statistical significance was determined with the

Student t test; $p < 0.05$ was considered as significant.

RESULTS

Development of DTH to *Mlm* antigens during the course of infection. The first series of experiments was performed to determine the time required by the foot pad-infected C3H mice to develop a positive DTH reaction to *Mlm* antigens, and to determine when the i.v.-infected mice became unresponsive to these antigens. Thus, a large number of mice were infected either subcutaneously (s.c.) into one hindfoot pad or i.v. with 1×10^7 *Mlm* bacilli and at 2-week intervals from weeks 3 to 19 post-infection, groups of 8 to 10 mice were challenged in the contralateral foot pad with a sonicated extract of *Mlm*. When infected s.c. (The Fig., A), the mice developed a cutaneous response that increased constantly from 5 to 13 weeks and then decreased slowly until the end of the observation period. The 24 hr and 48 hr response became statistically different from the background reaction at 5 and 9 weeks, respectively. In contrast, when infected i.v. (The Fig., B), the mouse mounted a very low and transient cutaneous response (the 48 hr response being not statistically different from the background reaction during the whole period of observation) that completely disappeared by week 19. These results were reproduced in a second similar experiment, and were in good agreement with those obtained by Patel in the B6D2F1 mice ([10]).



THE FIGURE. Development of the 24 hr (-----) and 48 hr (——) DTH responses in C3H mice infected subcutaneously (**A**) and intravenously (**B**) with 10^7 *Mlm*. Infected mice were challenged with the equivalent of 10^8 sonicated *Mlm* bacilli. Each point represents the mean ± S.E.M. of 8 to 10 mice. Horizontal shaded area indicates the range of the background reaction detected in normal mice 48 hr after challenge with 10^8 sonicated *Mlm*.

Based upon these results, we decided to elicit DTH in mice that received adoptive cell transfers at 5 weeks post-infection and to use the donors of spleen cells at 4 months after i.v. infection, that is, at the time they were completely immunodepressed.

Adoptive transfer of spleen-cell populations on the induction of DTH. Whole spleen-cell and T-cell populations from immunodepressed and normal mice, as controls, were transferred i.v. into syngeneic mice just prior to the hindfoot pad inoculation of 1×10^7 bacilli. Another control group, consisting of mice successively infected i.v. with 1.5×10^8 (that is, the number of contaminating bacilli per dose of transferred T-cell-enriched populations) and in the foot pad with 1×10^7 bacilli, was included in these experiments in an attempt to evaluate the effects of contaminating bacilli on DTH. Five weeks later, all of the experimental and control groups of mice were challenged in the contralateral foot pad with *Mlm* antigens and the foot pad thickness was measured 24 hr after challenge. As seen in Table 1, mice treated with the whole spleen cell and the T-cell-enriched populations from immunodepressed mice developed a significantly lower DTH reaction compared to either control mice treated with the corresponding normal cell populations or to the infected controls. The development of DTH was not affected to a significant extent in mice treated with the B cells or the plastic-adherent cell populations from immunodepressed mice (data not shown).

Adoptive transfer of spleen-cell populations on the expression of DTH. In these experiments, the various spleen-cell popu-

TABLE 1. *Effects of transferred whole spleen-cell and T-cell populations on the induction and the expression of DTH.*

Transferred cell populations[a]	Donor mice	Foot pad thickness (0.1 mm ± S.E.M.)[b]			
		Induction	p value[c]	Expression	p value[c]
Whole population	Normal	4.2 ± 0.2		5.1 ± 0.7	
	Depressed	1.2 ± 0.4	<0.01	1.2 ± 0.2	<0.005
Enriched T cells	Normal	3.2 ± 0.4		2.4 ± 0.2	
	Depressed	1.9 ± 0.3	<0.02	1.6 ± 0.2	<0.05
Infected controls[d]		3.9 ± 0.4		4.2 ± 0.4	

[a] Each mouse was injected i.v. with 1×10^7 whole spleen cells or enriched T cells from either normal or immunodepressed mice just prior to (induction) or 5 weeks after (expression) the inoculation in one hindfoot pad of 1×10^7 *Mlm* bacilli.

[b] Five weeks (induction) or 24 hr (expression) after cell transfers, the recipient mice (and the infected controls) were challenged in the contralateral foot pad with *Mlm* antigens. Foot pad thickness was evaluated 24 hr later and expressed in 0.1 mm units ± the standard error of the mean from 5 to 7 mice per group.

[c] When compared to the corresponding group of mice receiving whole spleen-cell or T-cell populations from normal donors.

[d] Infected controls consist of s.c. infected mice inoculated i.v. with 1.5×10^8 *Mlm* bacilli instead of spleen cells.

lations from either immunodepressed or normal mice were transferred i.v. to mice that had been infected 5 weeks previously with 1×10^7 *Mlm* bacilli, and the DTH reaction was elicited 24 hr after the adoptive transfer. When compared to mice transferred with normal cell populations, the level of the DTH reaction was significantly depressed in mice receiving the whole spleen-cell population and the T-cell-enriched population (Table 1) but not in mice receiving B lymphocytes and adherent cells (data not shown).

Specificity of suppressor cells. The specificity of *Mlm*-induced suppressor cells was tested against the DTH response to SRBC. Neither the induction nor the expression of DTH to SRBC was depressed by adoptive transfers of cells from *Mlm*-infected mice, implying that the *Mlm*-induced suppressor cells were specific in their action (data not shown).

Partial characterization of suppressor T cells. The T-cell-enriched population from immunodepressed mice was either irradiated at 2500 rads or treated with monoclonal anti-Lyt antibodies in an attempt to characterize the T-lymphocyte subsets that depressed the induction and the expression of DTH. The relative proportion of Lyt 1+ and Lyt 2+ in the T-cell-enriched preparation was, respectively, 51.6% and 26.4% for normal mice and 28.4% and 28.6% for *Mlm*-infected mice. The results of a representative experiment, depicted in Table 2, showed that the T-lymphocyte subset that depressed the induction of the DTH to *Mlm* antigens was radiosensitive and destroyed by treatment with both the anti-Lyt 1.1 and anti-Lyt 2.1 antisera and was C′. In contrast, the T-lymphocyte subset that depressed the expression of the DTH reaction was resistant to γ-irradiations and to the action of anti-Lyt 2.1 serum plus C′, but sensitive to the anti-Lyt 1.1 serum.

During this study, the survival time of mice transferred with the different cell preparations was observed in an attempt to evaluate the effect of suppressor cells upon anti-*Mlm* resistance. In general, a significant depression of the mean survival time was observed in mice transferred with the whole spleen cell, T-cell-enriched, and γ-irradiated populations, but not in the mice receiving B-cell and adherent-cell populations (data not shown).

DISCUSSION

The data presented in this paper show that C3H mice infected i.v. with 10^7 *Mlm* bacilli produced suppressor T cells, as demonstrated by adoptive transfer experiments, which inhibited the induction (afferent phase) as well as the expression (efferent phase) of DTH response to *Mlm* antigens. The *Mlm*-induced suppressor cells were antigenically specific in the sense that they did not affect the development or the expression

TABLE 2. *Partial characterization of T-suppressor cells involved in the induction and the expression of DTH reaction to* Mlm *antigens.*

Cell treatments[a]	Donor mice	Foot pad thickness (0.1 mm ± S.E.M.)[b]			
		Induction	p value[c]	Expression	p value[c]
None	Normal	3.2 ± 0.4		2.4 ± 0.2	
	Depressed	1.9 ± 0.3	<0.02	1.6 ± 0.2	<0.05
Irradiation	Normal	2.7 ± 0.4		2.6 ± 0.3	
	Depressed	2.6 ± 0.4	NS[d]	0.9 ± 0.3	<0.005
Anti-Lyt 1+	Normal	3.1 ± 0.3		2.9 ± 0.3	
	Depressed	3.7 ± 0.4	NS	2.9 ± 0.2	NS
Anti-Lyt 2+	Normal	3.2 ± 0.4		2.7 ± 0.2	
	Depressed	2.8 ± 0.3	NS	1.6 ± 0.2	<0.005

[a] T-cell-enriched populations from either normal or immunodepressed mice were adjusted to 1×10^7/ml. After γ-irradiation or anti-serum treatment, the cells were washed 3 times, adjusted to the original concentration, and transferred i.v. to mice just prior to infection (induction) or 5 weeks after infection (expression).

[b] Mice were challenged in one hindfoot pad with *Mlm* antigens 5 weeks (induction) or 24 hr (expression) after cell transfers. Foot pad thickness was measured 24 hr later. Each group was composed of 5 to 7 mice.

[c] When compared to the corresponding group of mice receiving whole spleen-cell or T-cell populations from normal donors.

[d] NS = not statistically significant.

of DTH against SRBC. The implication of specific suppressor T cells in the control of the DTH response in *Mlm*-infected mice is consistent with the observations made by several other investigators using various models in which the DTH reaction was induced with either nonreplicating antigens, allergens, or living infectious agents ([6]). Splenic T-suppressor cells that markedly inhibited the induction, but with a weaker effect on the expression of DTH to BCG antigens, have also been found in C3H/He mice when infected i.v. with the nonpathogenic BCG strain ([9]).

There are some indications that two different types of *Mlm*-induced suppressor cells (macrophage-like and T lymphocytes) inhibited several immune functions when assayed by *in vitro* techniques ([2, 14]), and among the suppressor T cells both the Lyt 1+, 2− and Lyt 1−, 2+ subsets from the spleen of *Mlm*-infected C57BL/6 mice had the ability to depress the mixed leukocyte reaction of normal splenocytes ([4]). These results contrasted with the present ones, since only the T-lymphocyte-enriched population and not the plastic-adherent population was active *in vivo,* at least in the control of the DTH reaction. Thus, the suppressor-cell populations or subpopulations that are active *in vitro* would differ from those active *in vivo.*

When the T-suppressor-enriched population was partially characterized by determining its sensitivity to γ-irradiation (2500 rads) and to monoclonal anti-Lyt antibodies and complement, a radiosensitive cell population expressing the Lyt 1+, 2+ phenotype was found to depress the induction of the DTH reaction; whereas a radioresistant cell population expressing the Lyt 1+, 2− phenotype had the capacity to depress the expression of the cutaneous reaction. A great deal of work has been carried out with various DTH models to determine the phenotype of T lymphocytes implicated in the adoptive transfer of DTH and in the regulation of this immune response. While a consensus seems to be reached concerning the phenotype of the effector cell (Lyt 1+, Lyt 2−, 3−, Ia−) responsible for DTH *in vivo* ([17]), the nature of the T-cell subpopulations involved in the suppression of DTH is more controversial. Nevertheless, the Lyt 1+, 2+ phenotype depressing the induction of DTH to *Mlm* antigens, as shown in the present study, is consistent with earlier results on the cell-surface phenotype of the nonspecific T-suppressor cells induced by the graft-versus-host reaction ([11]). Moreover, the Lyt 1+, 2− phenotype depressing the expression of DTH is the same as that of suppressor cells for DTH to the influenza virus ([7]) and *Leishmania tropica* ([3]). The fact that Lyt 1+, 2+ and Lyt 1+, 2− T-suppressor cells, respectively, depressed the induction and the expression of DTH to

Mlm antigens strongly suggests that the regulation of the afferent and the regulation of the efferent arms of DTH follow different pathways. The different populations of suppressor T cells in the *Mlm* model would differ from the first-order suppressor cells (TS_1) possessing the Lyt 1+, 2− phenotype and suppressing the induction, and from the second-order suppressor cells (TS_2) possessing the Lyt 1−, 2+ phenotype and suppressing the expression of DTH, as described in mice infected with *Cryptococcus neoformans* ([8]).

Previous results from this laboratory ([16]) had shown that the i.v. administration of BCG partially depressed the induction and the expression of DTH to BCG antigens. Thus, the suppressor effects on the DTH reaction, as reported in the present study, could be caused by the presence of *Mlm* bacilli contaminating the adoptively transferred cells and not by the presence of cells with suppressor activity. This possibility can be, at least in part, ruled out for the following reasons. First, the B-lymphocyte and the adherent-cell preparations used in adoptive transfers were contaminated with AFB to about the same (if not to a greater) extent as the T-lymphocyte preparations, and only the latter preparation possessed suppressor activity. Second, the transfer of normal spleen cells (10^7 cells) previously incubated for 4 hr with 1.5×10^8 *Mlm* bacilli did not depress the induction or the expression of DTH to *Mlm* antigens (data not shown). Finally, i.v. inoculation of culture supernatants prepared from spleen cells of immunodepressed mice and then filtered through a 0.2 μm-pore size membrane in order to eliminate the contaminating AFB had the ability to depress the efferent phase of DTH to *Mlm* antigens, the intensity of the local granulomatous reaction at the site of *Mlm* injection, and the mean survival time of recipient mice (data not shown). This last observation also implied that the *Mlm*-induced suppressor cells can elaborate active soluble factors, as found previously in other DTH models ([6]). The present data, therefore, strongly suggest that suppressor cells and/or suppressor factors play an active role in the control of DTH to *Mlm* antigens.

SUMMARY

Spleen cells from immunodepressed C3H mice, i.e., mice inoculated intravenously 4 months earlier with 1×10^7 *Mycobacterium lepraemurium* (*Mlm*) bacilli, were separated into different populations, and the T-cell-enriched population was treated further with gamma-irradiation or specific anti-Lyt antibodies plus complement. The cell populations obtained were then adoptively transferred to normal and *Mlm*-sensitized syngeneic mice in order to investigate whether or not suppressor cells regulate the delayed-type hypersensitivity (DTH) reaction to specific antigens. A radiosensitive cell population expressing the Lyt 1+, 2+ phenotype had the capacity to depress the induction (afferent phase) of DTH reaction. In contrast, a radioresistant cell population expressing the Lyt 1+, 2− phenotype possessed the capacity to depress the expression (efferent phase) of the cutaneous reaction. Thus, distinct populations of suppressor cells, each regulating a different phase of DTH, are induced in the spleen of *Mlm*-infected mice.

RESUMEN

Las células del bazo de ratones CEH inmunodeprimidos, i.e., ratones inoculados intravenosamente 4 meses antes con 1×10^7 *Mycobacterium lepraemurium* (MLM), se separaron en diferentes poblaciones y la población enriquecida en células T se trató posteriormente con irradiación gamma o con anticuerpos anti-Lyt más complemento. Las poblaciones celulares obtenidas fueron después transferidas adoptivamente a ratones singénicos normales y a ratones sensibilizados con MLM para investigar si las células supresoras regulaban o no la hipersensibilidad de tipo tardío (HTT) contra antígenos específicos. Una población de células radiosensibles con el fenotipo Lyt 1+, 2+, tuvo la capacidad de deprimir la inducción (fase aferente) de la reacción de HTT. En contraste, una población celular radioresistente con el fenotipo Lyt 1+, 2−, poseyó la capacidad de deprimir la expresión (fase eferente) de la reacción cutánea. Así, en el bazo de los ratones infectados con MLM se inducen distintas poblaciones de células supresoras, cada una capaz de regular diferentes fases de la HTT.

RÉSUMÉ

Des cellules spléniques provenant de souris C3H immunodéprimées ont été séparées en différentes populations. Ces souris avaient été inoculées quatre mois

auparavant par voie intraveineuse, avec 1×10^7 bacilles de la lèpre murine, *Mycobacterium lepraemurium* (MLM). La population enrichie en cellules-T a ensuite été soumise à une irradiation par rayons gamma, de même qu'à des anticorps spécifiques anti-Lyt accompagnés de complément. Les populations cellulaires ainsi obtenues ont alors été tranférées à des souris syngéniques, soit normales soit sensibilisées par *M. lepraemurium*, dans le but d'explorer une éventuelle régulation, par les cellules suppressives, de la réaction d'hypersensibilité retardée à des antigènes spécifiques. Une population cellulaire radiosensible exprimant le phénotype Lyt 1+, 2+ est capable de déprimer l'induction de la réaction d'hypersensibilité retardée (phase afférente). Par contre, une population cellulaire radiorésistante exprimant le phénotype Lyt 1+, 2− a le pouvoir de déprimer l'expression de la réaction cutanée (phase efférente). On en conclut que des populations distinctes de cellules suppressives, chacune contrôlant une phase différente de la réaction d'hypersensibilité retardée, peuvent être entraînées à se développer dans la rate de souris infectées par le bacille de la lèpre murine.

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REFERENCES

1. Brett, S. J. T-cell responsiveness in *Mycobacterium lepraemurium* infections in a "resistant" (CBA) and a "susceptible" (BALB/c) mouse strain. Cell. Immunol. **89** (1984) 132–143.
2. Bullock, W. E., Carlson, E. M. and Gershon, R. K. The evolution of immunosuppressive cell populations in experimental mycobacterial infection. J. Immunol. **120** (1978) 1709–1716.
3. Dhalimal, J. S., Liew, F. Y. and Cox, F. E. G. Specific suppressor T cells for delayed-type hypersensitivity in susceptible mice immunized against cutaneous leishmaniasis. Infect. Immun. **49** (1985) 417–423.
4. Hoffenbach, A., Lagrange, P. H. and Bach, M.-A. Surface Lyt phenotype of suppressor cells in C57BL/6 mice infected with *Mycobacterium lepraemurium*. Clin. Exp. Immunol. **54** (1983) 151–157.
5. Klimetzek, V. and Remold, H. G. The murine bone marrow macrophage, a sensitive indicator cell for murine migration inhibitory factor and a new method for their harvest. Cell. Immunol. **53** (1980) 257–266.
6. Liew, F. Y. Regulation of delayed-type hypersensitivity to pathogens and alloantigens. Immunol. Today **3** (1982) 18–23.
7. Liew, F. Y. and Russell, S. M. Delayed-type hypersensitivity to influenza virus. Induction of antigen specific suppressor T cells for delayed-type hypersensitivity to hemagglutinin during influenza virus infection in mice. J. Exp. Med. **151** (1980) 799–814.
8. Murphy, J. W. Effects of first-order *Cryptococcus*-specific T-suppressor cells in induction of cells responsible for delayed-type hypersensitivity. Infect. Immun. **48** (1985) 439–445.
9. Nakamura, R. M. and Tokunaga, T. Induction of suppressor T cells in delayed-type hypersensitivity to *Mycobacterium bovis* in low-responder mice. Infect. Immun. **28** (1980) 331–335.
10. Patel, P. J. Antibacterial resistance in mice infected with *Mycobacterium lepraemurium*. Clin. Exp. Immunol. **45** (1981) 654–661.
11. Pickel, K. and Hoffman, M. K. The Ly phenotypes of suppressor T cells arising in mice subjected to graft-vs-host reaction. J. Exp. Med. **145** (1977) 1169–1175.
12. Poulter, L. W. and Lefford, M. J. Development of delayed-type hypersensitivity during *Mycobacterium lepraemurium* infection in mice. Infect. Immun. **17** (1977) 439–446.
13. Shepard, C. C. and McRae, D. H. A method for counting acid-fast bacteria. Int. J. Lepr. **36** (1968) 78–82.
14. Turcotte, R. Suppressor cells in experimental murine leprosy. Int. J. Lepr. **46** (1978) 358–363.
15. Turcotte, R. Influence of route of *Mycobacterium lepraemurium* infection on susceptibility to mouse leprosy and on lymphoblastic transformation. Infect. Immun. **28** (1980) 660–668.
16. Turcotte, R. and Forget, A. Cutaneous unresponsiveness to *Mycobacterium bovis* BCG in intravenously infected mice. Infect. Immun. **41** (1983) 453–461.
17. Vadas, M. A., Miller, J. F. A. P., McKenzie, I. F. C., Chism, S. E., Shen, F. W., Boyse, E. A., Gamble, J. R. and Whitelow, A. M. Ly and Ia antigen phenotypes of T cells involved in delayed-type hypersensitivity and in suppression. J. Exp. Med. **144** (1976) 10–19.
18. Wysocki, L. J. and Sato, V. L. "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. U.S.A. **75** (1978) 2844–2848.