LITERATURE CITED

1. E. A. Bazanova, A. F. Panasyuk, and I. M. Lyampert, Vestn. Akad. Med. Nauk SSSR, No. 7, 39 (1986).
2. E. A. Bazanova, É. V. Gnezditskaya, N. A. Borodiyuk, and E. Yu. Pyt'eva, Zh. Mikrobiol., No. 6, 86 (1989).
3. E. A. Bazanova, É. V. Gnezditskaya, N. A. Borodiyuk, and I. M. Lyampert, Zh. Mikrobiol., No. 1, 80 (1990).
4. N. A. Borodiyuk, I. M. Lyampert, I. N. Mikhailova, et al., Zh. Mikrobiol., No. 6, 77 (1985).
5. I. M. Lyampert, Immunologiya, No. 4, 5 (1988).
6. I. M. Lyampert, E. I. Drobyshevskaya, E. V. Ryzhikova, et al., Progress in Science and Technology. Series: Immunology [in Russian], Vol. 22, Moscow (1988), pp. 43-67.
7. E. V. Ryzhikova, V. V. Spirina, V. L. Cherkasov, et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 45 (1986).
8. J. E. Coligan, T. Y. Kindt, and R. M. Krause, Immunochemistry, **15**, 755 (1978).
9. É. V. Gnezditskaya and L. V. Beletskaya, in: Basic Concepts of Streptococci and Streptococcal Diseases, S. E. Holm and B. P. Christensen (eds.), Chertsey, England (1982), pp. 256-257.
10. B. F. Haynes, Adv. Immunol., **36**, 87 (1984).
11. I. M. Lyampert, L. V. Beletskaya, N. A. Borodiyuk, et al., Immunology, **31**, 47 (1976).
12. I. M. Lyampert, M. N. Smirnova, and E. A. Bazanova, Cell. Immunol., **52**, 325 (1980).
13. B. N. Manjula and V. A. Fischetti, J. Immunol., **124**, 261 (1980).

CELLULAR MECHANISMS OF SUPPRESSION OF T LYMPHOCYTE PROLIFERATION BY LUNG CELLS IN EXPERIMENTAL TUBERCULOSIS

I. G. Kramnik, A. M. Moroz, and A. S. Apt

UDC 616.24-002.5-092.9-07:616.155.32

KEY WORDS: lung cells; immunosuppression; tuberculosis

The course of chronic infections diseases caused by intracellular pathogens, including *Mycobacterium tuberculosis*, in laboratory animals and man is accompanied by the development of suppression of the immune response [1, 11]. It is also known that immunocompetent lung cells in healthy animals have a suppressor action on certain immunologic reactions [7, 8]. However, the effects of cells of the lung, i.e., the organ of specific localization of tuberculus infection, on the immune response in tuberculosis have not been studied, and this is particularly true of the relations between specific and nonspecific immunosuppression in this infection.

Previously [2], the writers described a high level of suppressor activity of cells isolated from the interstitial tissue of the lung of mice infected with the virulent strain *M. tuberculosis* H37Rv. Suppression took place through the action of cells adherent to plastic and to nylon wadding, and characterized, besides by nonspecific activity, by a marked antigen-specific component, because small doses of suppressor cells inhibited only proliferation of T lymphocytes immune to mycobacterial antigens, in response to stimulation by tuberculin (PPD). High doses of lung cells suppressed the response to other antigens also, suggesting the existence of at least two suppressor mechanisms in foci of tuberculosis infection. In this paper we described the results of a study of the cellular mechanisms of immunosuppression in mice with tuberculosis.

Laboratory of Experimental Immunogenetics, Central Research Institute of Tuberculosis, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. G. Khomenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 172-176, August, 1990. Original article submitted December 15, 1989.

 0007-4888/90/0008-1074$12.50

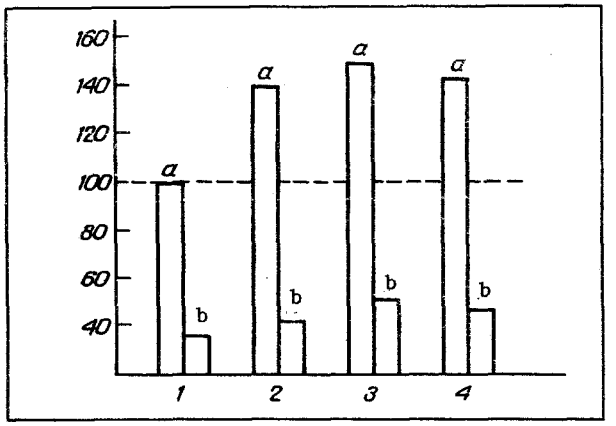

Fig. 1. Lymphocytes isolated from lungs of infected mice have no suppressor action on proliferative response of immune lymphocytes to PPD. a) Proliferation of cells of immune lymph node in presence of PPD, b) without antigen, 1) without lung cells, 2) in presence of fraction of lung cells enriched with lymphocytes by centrifugation on Percoll gradient (100,000 cells per well), 3) in presence of lung cells, removed from nylon wadding (100,000 cells per well), 4) in presence of T lymphocytes isolated from lung and stimulated in vitro for 72 h in presence of 10% supernatant containing IL-2, treated with mitomycin C.

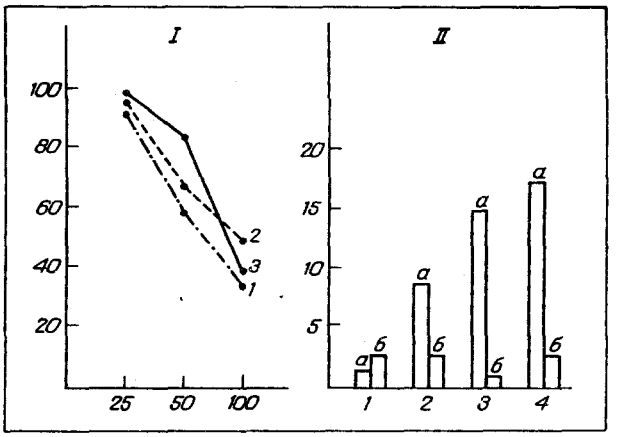

Fig. 2. Removal of T and B lymphocytes from population of interstitial cells of the lung does not abolish the suppressor effect. I) Abscissa, number of lung cells (in thousands per well) treated with: complement (1), anti-Thy 1.2-serum with complement (2), cells carrying surface immunoglobulin (B lymphocytes) removed by panning (3). II) Removal of cells undertaking phagocytosis of iron carbonyl potentiates proliferative response of lung cells to EPD; a) proliferation of lung cells in presence of PPD (10 μg/ml), b) without antigen. 1) Unfractionated lung cells; 2) lung cells not adherent to plastic; 3) lung cells not adherent to plastic and nylon wadding; 4) lung cells not undertaking phagocytosis of iron carbonyl.

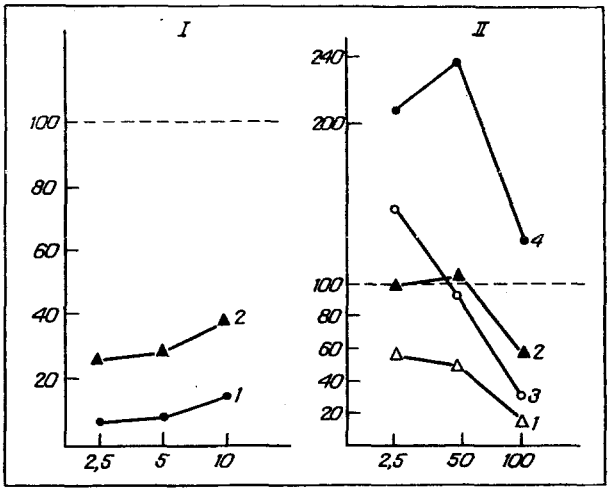

Fig. 3. Indomethacin does not abolish suppression of the response of immune lymphocytes to PPD by adherent lung cells. I: 1) 50,000 adherent cells per well, 2) 25,000 adherent cells per well. II) Different action of indomethacin (in μg/ml) in a dose of 10 μg/ml on suppression of antigen-specific proliferation of lymphocytes immune to antigens of *M. tuberculosis* and *Staphylococcus* by infected lung cells (thousands per well). 1) Lymphocytes immune to mycobacterial antigens, 2) the same in the presence of indomethacin, 3) lymphocytes immune to staphylococcal antigens, 4) the same in the presence of indomethacin. Ordinate, response, in per cent.

EXPERIMENTAL METHOD

Mice of the inbred line CBA/Sto aged 3-4 months, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, were used in the experiments. Infection of the mice and preparation of suspensions of interstitial lung cells were described previously [2].

A suspension of lung cells was divided into fractions of cells differing in density by centrifugation on a stepwise Percoll gradient (Pharmacia). For this purpose, a 100% isotonic solution of Percoll was prepared by the addition of one part of 10 times Dulbecco phosphate-salt buffer without Ca^{2+} and Mg^{2+} (PBS, from Flow Laboratories) to 9 parts of Percoll. Twenty, 35, 50, and 70% solutions of Percoll were obtained by mixing the corresponding amounts of 100% isotonic Percoll with a 0.5% solution of bovine serum albumin (Sigma) in PBS. Next (50-60) $\times 10^6$ lung cells were washed in calcium-free medium, and resuspended in 5 ml of 100% Percoll, after which, 70, 50, 35, and 20% solutions of Percoll in volumes of 5 ml were layered on them consecutively. After centrifugation (4°C, 30 min, 600*g*) the cell rings thus obtained were harvested with a finely tapering Pasteur pipet and the cell fractions were washed 3 times to remove Percoll.

Phagocytic cells were removed after incubation of the suspension of lung cells (5×10^6/ml) with iron carbonyl (25 mg/ml) for 1 h at 37°C, by means of a permanent magnet, the incubation in the magnetic field being repeated 3 times, for 5 min each time.

Cells carrying surface immunoglobulin (Ig^+, B lymphocytes) were removed by incubating the suspension of lung cells (5×10^6/ml) on plastic Petri dishes, coated with affinity-purified rabbit antibodies against mouse immunoglobulins (Calbiochem) at 4°C, twice for 1 h each time.

T lymphocytes were removed from the suspension of lung cells by treating the cells with antiserum against Thy 1.2 marker for 1 h at 4°C, and then with rabbit complement (Cederlane) in a dilution of 1:10 for 45 min at 37°C, after which the cells were washed off 3 times.

TABLE 1. Lung Cells of Mice Infected with Tuberculosis Suppress Proliferative Response of Unfractionated Lymph Node Cells but Do Not Suppress Response of T Lymphocytes Nonadherent to Nylon Wadding

Lymph node cells	Pressors, number of cells per well ($\times 10^{-3}$)	Proliferation		IS	Per cent of suppression
		with antigen (PPD)	without antigen		
Unfractionated	—	31,4±3,5	4,2±0,6	7,8	—
	40	5,8±0,7	3,7±0,5	1,6	93
	10	9,8±1,3	2,3±0,3	4,3	73
Nonadherent to nylon wadding	—	6,1±0,9	0,9±0,1	6,8	—
	40	6,0±1,0	1,7±0,1	3,5	17
	10	5,0±0,8	0,9±0,2	5,6	21

Preparation of lung T cells on columns with nylon wadding and of the immune lymphocytes from lymph nodes, and the conduct of the proliferative tests in vitro were described previously [2].

In some experiments populations of suppressor cells were treated with mitomycin C (Kyuo) in a concentration of 25 μg/ml at 37°C for 30 min. Indomethacin (Sigma) was dissolved in ethanol in a concentration of 10 mg/ml and added to the culture medium to assess the proliferative response in a final concentration of 1-10 μg/ml.

Intracellular mycobacteria were detected by staining methanol-fixed adherent lung cells with 0.1% auramine 00 for 20 min at room temperature.

EXPERIMENTAL RESULTS

A previous investigation [2] showed that removal of cells belonging to the interstitial cell population of the lung infected mice, adherent to plastic, leads to partial restoration of the proliferative response to the specific antigen (PPD). For complete restoration of the response additional purification was required from cells adherent to nylon wadding. Since B cells and some subpopulation of T lymphocytes also adhere, besides macrophages, to nylon wadding [10], it was not clear whether these lung cells also take part in suppression.

By fractionating the lung cells on a stepwise Percoll gradient fractions of relatively dense cells (ρ = 1.065-1.077), considerably enriched with lymphocytes, could be obtained [9]. Combined culture of these cells, nonadherent to plastic, when treated with mitomycin C, with cells of immune lymph nodes in the presence of antigen not only did not suppress but, on the contrary, led to potentiation of the response to specific antigen (Fig. 1). Lung cells adherent to nylon wadding and removed from it mechanically, according to microscopic examination, were mainly lymphocytes and likewise possessed no suppressor activity (Fig. 1). Treatment of unfractionated lung cells with antiserum against the T-cell marker Thy 1.2 and complement did not abolish the suppressor action of these cells (Fig. 2a). Removal of cells carrying surface immunoglobulin from the population of lung cells by adsorption on plastic, covered with antibodies to mouse immunoglobulins, by the direct panning method, likewise did not abolish the suppressor effect (Fig. 2a). Moreover, the T-lymphocyte population of the lung, purified on a column with nylon wadding and stimulated by PPD in culture in vitro for 3 days in the presence of the supernatant culture fluid containing IL-2, likewise was unable to suppress the response of immune lymph node cells to PPD (Fig. 1). Thus, in these experiments the view that the suppressor cells in the lung are lymphocytic in origin was not confirmed experimentally.

To prove the hypothesis that the principal effectors of suppression are macrophage-like cells additional experiments were undertaken. Culture for 24 h led to detachment of nonmacrophagal cells, which had been adherent during the first hours, from plastic [3]. In our experiments culture of adherent cells for 24 and 48 h, followed by washing the monolayer to remove detached cells, did not abolish the suppressor action of firmly adherent cells. The population of lung cells purified from cells adherent to plastic contained up to 10% of macrophage-like cells, evidently incapable of adhesion. Removal of cells undertaking phagocytosis of iron carbonyl from this population with the aid of a magnet led to considerable potentiation of the proliferative response of the nonphagocytic lung cells to PPD (Fig. 2b).

It can be concluded from the facts described above that phagocytic lung cells adherent to plastic are the main type of suppressors activated during the development of tuberculosis infection.

The suppressor action of monocytes and macrophages is often linked with the production by these cells of prostaglandin E_2 (PGE_2), which inhibits, in particular, IL-2 secretion [14]. In the experiment illustrated in Fig. 3 the effect of indomethacin, a drug inhibiting prostaglandin synthesis [4], on inhibition of the proliferative response by lung cells was investigated. Over a wide range of concentrations indomethacin significantly reduced nonspecific suppression of the response of immune lymphocytes to

TABLE 2. Activity of Suppression Factors Obtained by Combined Culture of Lymphocytes Immune to Antigens of *M. tuberculosis* with Cells Adherent to Plastic and Isolated from Lungs of Mice Infected with Tuberculosis

Antigen or mitogen	Suppression factor	Proliferation		IS	Per cent of suppression
		with antigen	without antigen		
PPD	—	11,2±1,6	2,5±0,4	4,4	—
	A	6,0±0,9	2,4±0,5	2,3	59
	B	3,8±0,7	1,8±0,4	2,1	76
Con A	—	27,5±1,8	2,5±0,4	11	—
	A	77,5±5,5	2,5±0,4	31	—283
	B	62,5±2,2	1,8±0,6	35	—227
PPD	—	17,6±2,1	2,4±0,4	7	—
	G(100)	24,0±5,2	5,1±0,7	4,8	—25
	C(40)	30,7±4,5	12,4±0,9	2,5	—22

Legend. A) Supernatant obtained after 72 h of culture of adherent lung cells, B) supernatant obtained after 72 h of culture of adherent lung cells together with immune lymphocytes, C) immune lymphocytes isolated from cultures after combined culture with adherent lung cells in the presence of PPD for 72 h (thousands per well).

staphylococcal antigen, but had a much weaker effect on suppression of the specific response to PPD, although with the low dose of suppressor cells used, in the latter case also a component of suppression was detected, evidently due to PGE_2. These experiments confirm the dual character of suppression of the immune response by lung cells of infected mice. The nonspecific component of suppression is largely connected with the production of prostaglandins by lung macrophages, but in addition there is a specific mechanism, independent of prostaglandins. Since specific suppression is not connected with the T cells of the lung, for it was not abolished by antibodies against Thy 1.2 (see above), experiments were carried out to detect the possible involvement of cells of the lymph nodes themselves in suppression.

According to the results of our microscopic investigation, among cells of the infected lung adherent to plastic, 2-8% of them contained intracellular mycobacteria. It can be postulated that if these cells carry mycobacterial antigens on the cell surface and possess antigen-presenting function, the addition of immune T lymphocytes to them ought to induce activation and proliferation of the latter. However, the addition of lymphocytes immune to *M. tuberculosis* to the adherent lung cells did not lead to proliferation of antigen-specific cells but, on the contrary, it greatly reduced their spontaneous proliferation (Table 1), i.e., activity of the suppressor cells was preserved. If, however, the same population of lung cells adherent to plastic was cultured, not with the unfractionated population of lymph node cells, but with T lymphocytes purified on nylon wadding, neither the spontaneous proliferation nor the weak but significant response of these cells to PPD was depressed (Table 1). Thus, for the realization (at least in its full range) of the suppressor action by macrophages, the population of responding cells must contain cells adherent to nylon wadding. Possibly these cells participate in antigen-specific interaction with macrophages of the lung, which is essential for manifestation of suppressor activity toward mycobacterial antigens.

The possible mechanism of antigen-specific suppression could be induction by macrophages of antigen-specific suppressor T cells in the cell population of immune lymph nodes. However, lymph nodes isolated from cultures after combined culture for 48-72 h with adherent lung cells in the presence of PPD (i.e., not adherent to plastic during the period of culture) and treated with mitomycin C did not suppress, but stimulated the proliferative response of immune lymphocytes to PPD (Table 2). Thus, in this experiment the suggestion that induction takes place in the responding population of suppressor cells was not confirmed.

Suppressor action on the responding cell population can take place either by direct intercellular contact or by secretion of factors. Investigation of the culture fluid obtained during combined culture of immune lymphocytes and lung cells of infected mice, adherent to plastic, in the presence of PPD demonstrated the ability of factors contained in it to considerably depress the proliferative response of the immune cells to PPD (Table 2). Conversely, during culture of the same lymphocytes with con A (2.5 μg/ml) stimulation of the proliferative response was observed on the addition of the same culture fluid (Fig. 2). On the one hand this result may be evidence of the antigenic specificity of the factor responsible for suppression present in the medium. On the other hand, it may be that processes of activation of T lymphocytes by con A and by specific antigen differ both in their threshold of activation and depending on the presence of different cytokines. This second hypothesis is confirmed by the analogous data on suppression and stimulation of the proliferative response obtained on other models of experimental infections.

For instance, in [5], during an investigation of macrophages isolated from hepatic granulomas of mice infected with *Schistosoma mansoni*, the culture fluid of these macrophages was found to contain interferons α and β, as well as IL-1, which suppress the response to schistosomal antigens and to other protein antigens, but stimulate the response to con A. According to data in [11], granulomas formed in the lungs of mice after intratracheal injection of BCG contain IL-1, increased production of which by monocytes may lead, not to stimulation, but to antigen-specific suppression in tuberculosis in man [6]. Recently, the participation of β growth-transforming factor, synthesized by activated bacterial products by macrophages, has attracted considerable attention [15].

Since mechanisms such as inhibition of expression of receptors to IL-2 by secreted factors [14] and synthesis of IL-2 itself [12] also participate in suppression of the T-cell response in infections, the question of the role of cytokines in specific and nonspecific suppression of the immune response in tuberculosis requires further investigation.

LITERATURE CITED

1. M. M. Averbakh, A. M. Moroz, A. S. Apt, and B. V. Nikonenko, The Immunogenetics of Infectious Diseases [in Russian], Moscow (1985).
2. I. B. Kramnik, A. S. Apt, and A. M. Moroz, Byull. Éksp. Biol. Med., No. 7, 77 (1990).
3. J. Berit and M. Berit, Acta Path. Microbiol. Immunol. Scand., **95C**, 233 (1987).
4. E. Cillary, F. Y. Liew, and R. Lelchuk, Infect. Immun., **54**, 386 (1986).
5. D. E. Elliot, V. F. Righthand, and D. L. Boros, J. Immunol., **138**, 2653 (1987).
6. J. J. Ellner and R. S. Wallis, Rev. Infect. Dis., **11**, Suppl. 2, 455 (1989).
7. D. A. Ferrick and H. B. Herscovitz, Cell. Immunol., **116**, 183 (1988).
8. P. G. Holt, A. Degebrodt, C. O'Leary, et al., Clin. Exp. Immunol., **62**, 586 (1985).
9. P. G. Holt, A. Degebrodt, T. Venaille, et al., Immunology, **54**, 139 (1985).
10. E. Kedar and M. Schwarthbach, Cell. Immunol., **43**, 326 (1979).
11. K. Kobayashi, C. Allred, S. Cohen, and T. Yoshida, J. Immunol., **134**, 358 (1985).
12. K. Kobayashi, C. Allred, and T. Yoshida, Immunobiology, **178**, 329 (1989).
13. S. L. Kunkel, S. W. Chensue, and S. H. Phan, J. Immunol., **136**, 186 (1986).
14. M. Sileghem, A. Darji, L. Remels, et al., Eur. J. Immunol., **19**, 119 (1989).
15. S. M. Wahl, D. A. Hunt, G. Bansal, et al., J. Exp. Med., **168**, 1403 (1988).