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Regulation of T-cell proliferative responses by cells from solid lung tissue of *M. tuberculosis*-infected mice

A. S. APT, I. B. KRAMNIK & A. M. MOROZ *Experimental Immunogenetics Laboratory, Central Institute for Tuberculosis, Moscow, USSR*

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SUMMARY

We have studied proliferative responses to mycobacterial antigen preparation (PPD) and to non-specific stimuli of interstitial cells from the lungs of *Mycobacterium tuberculosis*-infected CBA mice. PPD-reactive lymphocytes appeared in the lung wall tissue in the course of chronic infection, but their proliferative capacity was totally inhibited by the lung macrophages. The latter were also able to suppress the proliferation of immune lymph node T cells. The mechanism of suppression clearly had two components, one being infection-specific and the other non-specific. Non-specific suppression was mediated mainly by prostaglandin E(PGE), whereas the specific mechanism showed only a weak influence of PGE and depended on the presence of I-J$^+$ Lyt-2$^-$ nylon-wool-adherent cells in the responder population. Interstitial lung T or B lymphocytes were not involved in specific suppression.

INTRODUCTION

The development and regulation of immune responses in organs which represent an interface between the internal homeostasis and environment, such as gut, lung, or urogenital tract, differ from those in other anatomical sites. A high load of pathogens and other external antigens on the mucosal surface requires the host to neutralize the continuous inflow of pathogens by local inflammatory and immunological reactions. On the other hand, a high level of these reactions may well lead to autoimmunity or allergy, reflected by a very high incidence of respiratory and urogenital infectious diseases as well as respiratory allergic states in human populations. Attempts to avoid this unavoidable contradiction in evolution are, presumably, reflected by the fact that at least some lymphocyte populations occupying the interface are armed with very specific recognizing structures: IgA B cells and polymorphic $\gamma\delta$ T-cell receptor (TcR) T cells.[1]

Studies on the peculiarities of immune response in lungs were limited for a long time by the exclusive use of bronchoalveolar lavage (BAL) cells. The pioneering work of P. Holt and his colleagues[2,3] on enzymatic disruption of lung tissue resulted in the development of methods for work with interstitial lung cells. These authors found that the alveolar interstitial macrophages were highly suppressive to T-lymphocyte activation and proliferation.[4–6]

Subpopulations of immunocompetent cells from murine lungs have been characterized in some detail as to their phenotype and functional activity,[7–9] and the role of murine BAL macrophages in regulation/suppression of immune responses was documented for definite test systems (see, for example, refs 10 and 11). However, little is known about the regulation of the immune response of murine interstitial lung cells, and neither the specific local immune responses to pathogens nor the influence of cells from infected lungs on the infection-specific or non-specific responsiveness of immunocompetent cells from other anatomical sites have been examined. With respect to infections, tuberculosis is of particular interest, because it was shown that the development of this lung-associated disease in mice and humans is accompanied by severe systemic immunosuppression,[12–15] although mice infected i.v. with a high dose of *Mycobacterium tuberculosis* are not a precise model for pulmonary disease in man.

In this work we have investigated immunological activities of interstitial lung cells from *M. tuberculosis*-infected and normal mice. It is shown that macrophages from infected lungs suppressed proliferative T-cell responses to various stimuli, and especially to mycobacterial antigens, much more effectively than macrophages from normal lungs. Immunosuppression of response against a mycobacterial antigenic preparation (PPD) by macrophages from infected lungs was clearly characterized by two components: antigen-specific and non-specific, the latter mediated, at least in part, by prostaglandin E (PGE). Some possible cellular mechanisms providing the antigen-specific component of suppression were also investigated and are discussed in this paper.

MATERIALS AND METHODS

Infection and immunization of mice

Female CBA/Sto mice, obtained from the Animal Breeding Farm 'Stolbovaya', Moscow District, USSR, were 3–4 months

Correspondence: Dr A. S. Apt, Experimental Immunogenetics Laboratory, Central Institute for Tuberculosis, Moscow 107564, USSR.

of age at the time of infection. Mice were injected i.v. with 12 μg (about 10^5 CFU) of a virulent live H37Rv strain of *M. tuberculosis* suspended in 0·5 ml of sterile saline. Mycobacteria were harvested from 3-week cultures on Loevenstein–Jensen medium suspended in saline containing 0·1% of bovine serum albumin (BSA) and 0·05 per cent of Tween-20 at 1 mg/ml, and stored at −70° until used. Mice were used at 3–6 weeks after infection, while their median survival time exceeded 9 weeks.

To obtain immune lymphocytes, mice were injected into footpads with 40 μl of either Freund's complete adjuvant (FCA) containing heat-killed *M. tuberculosis* H37Ra (Sigma, St Louis, MO) or 100 μg/ml ovalbumin (OVA; Sigma) in Freund's incomplete adjuvant (IFA; Sigma), or a preparation of radiation-killed *Staphylococcus aureus* (5×10^6 microbes/ml) in IFA. Antigenic preparations of *S. aureus* were a kind gift Dr M. M. Averbakh. Popliteal lymph nodes were removed on Days 9–14 after immunization; single cell suspensions were prepared and washed twice with 199 medium containing 2% foetal calf serum (FCS), 10 mM HEPES and antibiotics (Flow, McLean, VA).

Antibodies

Affinity-purified rabbit anti-mouse Ig antibodies were purchased from Calbiochem, La Jolla, CA. Hyperimmune antisera, CBA versus AKR against the Thy-1.2 marker and B10.A(3R) versus B10.A(5R) against the I-J^k determinant, were produced locally.[12,13] Hybridomas H35-17.2 and 10-2-16 were a kind gift from Dr B. D. Brondz and their supernatants were used as a source of monoclonal antibodies (mAb) to Lyt-2^{16} and I-A^{k17} antigens, respectively. For cytotoxic treatments and blocking experiments, hybridoma supernatants were used at the final concentration 1:20; anti-Thy-1.2 antiserum, 1:30; anti-I-J-^{k} antiserum, 1:5. Low-toxic M complement (Cedarlane Laboratories, Westbury, NY) was utilized in cell-depletion experiments according to the recommendations of the producer.

Lung cell suspension

The method employed was enzymatic disruption, as described by Holt *et al.*,[3] with some identifications. Mice were anaesthetized with an overdose of barbiturate and blood vessels were washed out by i.v. infusion of 7 ml of Hank's balanced saline solution (HBSS) containing 100 U/ml penicillin, 100 μg/ml streptomycin and 10 U/ml heparin via retro-orbital venous plexus. BAL with 1·5 ml of warm saline containing antibiotics via cannulated trachea with subsequent aspiration of liquid was repeated eight times. After these procedures, resulting in washing away free blood and BAL cells from the tissue, lungs were removed from thorax, additionally washed twice with HBSS with antibiotics, and sliced into pieces 1–2 mm^3. Slices were incubated with shaking for 1·5 hr at 37° in 20 ml of medium L-15 supplemented with 20 mM HEPES, 1% FCS, 50 U/ml kanamycin (all components from Flow), 260 U/ml collagenase/ (Boehringer, Mannheim, Germany) and 50 μg/ml DNAase (USSR). A single-cell suspension was obtained after repeated pipetting and filtration through a stainless steel net. The cells were washed three times with 199 medium with 1% FCS and 20 mM HEPES at 150 *g*, filtered through a thin cotton wool plug, and resuspended in the full culture medium, i.e. RPMI-1640 supplemented with 5% FCS, 10 mM HEPES, 4 mM L-glutamine, 1% non-essential amino acids, 2·2 mM pyruvate, 5×10^{-5} 2-mercaptoethanol and 50 U/ml kanamycin (all components from Flow). The viability of cells, as determined by trypan blue exclusion, was 80–90%.

Separation of lung cells

The following procedures were used to obtain lung cell suspensions enriched for or depleted of definite populations of immunocompetent cells.

Plastic-adherent cells were removed by incubation of cell suspensions in culture medium on 90-mm Petri dishes (2·5–3×10^7 cells per dish) for 2 hr at 37° in 5% CO_2 atmosphere. As indicated in the Results, incubation lasted for 24 hr in some experiments. The adherent cells were collected by vigorous pipetting after incubation of monolayer with cold Versene solution (0·02% EDTA) for 30 min.

Populations depleted of nylon-wool-adherent (NWA) cells (enriched for T lymphocytes) were obtained after passage of 5×10^7 plastic non-adherent lung cells or immune lymph node cells through a 10-ml syringe column containing 0·7 g of nylon-wool (Fenwall). After incubation of a column for 1 hr at 37°, cells were eluted with warm culture medium.

Lung cells were also separated by centrifugation on Percoll density gradient. $5–6 \times 10^7$ lung cells were suspended in 5 ml of 100% isotonic Percoll solution (Pharmacia, Uppsala, Sweden) and then 1·5 ml of 70%, 50%, 35% and 20% Percoll solutions were successively overlaid. After centrifugation for 30 min at 4°, 600 *g*, the rings containing separate cell fractions were collected separately and washed three times before use.

Phagocytes were removed from populations of lung cell by incubation with carbonyl iron (Sigma) for 1 hr at 37° (5×10^6 cells, 25 mg iron per 1 ml of culture medium), with subsequent sedimentation with a magnet.

Depletion of cells carrying Thy-1.2, Lyt-2 or I-J antigens was performed by two-step cytotoxic reaction with antibodies (1 hr, 4°) and complement (45 min, 37°).

Proliferative responses

4×10^5 lung or lymph node cells were cultured in 0·2 ml of full culture medium in the well of a flat-bottomed plate (Nunc, Roskilde, Denmark) for 48–96 hr (last 6 hr with 1 μCi of [^{3}H]thymidine) in the CO_2 incubator. Cells were stimulated with either 10 μg/ml PPD (Statum Seruminstitut, Copenhagen, Denmark), 20 μg/ml ovalbumin (OVA, Sigma), 10 μg/ml cytoplasmic fraction of *S. aureus*, or 5 μg/ml concanavalin A (Con A; Pharmacia, Uppsala, Sweden). Non-stimulated wells served as controls. All variants of cultures were performed in triplicate. Cultures were terminated by harvesting the well's contents onto fibreglass filters, and [^{3}H]thymidine uptake was measured in a liquid scintillation counter.

The suppressive effect

This was measured by co-culturing of 4×10^5 immune lymph node cells with various numbers of fractionated or unfractionated lung cells. In some experiments the latter were pretreated with 25 μg/ml mitomycin C (Kyuo) at 37° for 30 min to abrogate their possible proliferation. Indomethacin (Sigma) solution in ethanol (10 mg/ml) was added to cultures at a final concentrations of 1–10 μg/ml.

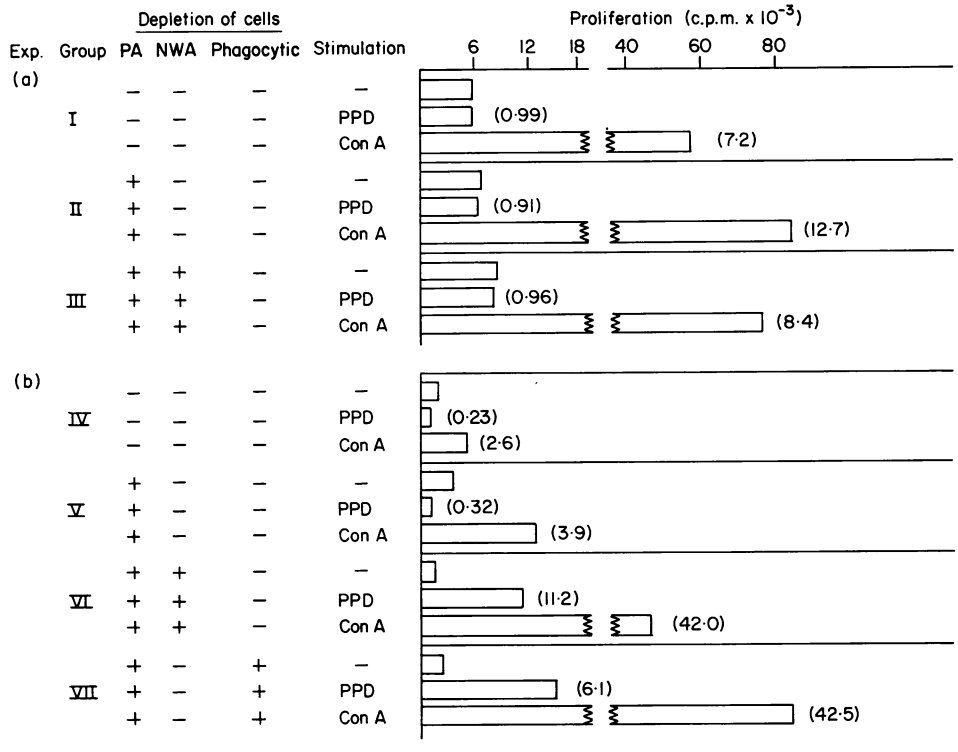

Figure 1. Proliferative responses of lung cells from normal (a) and tuberculosis-infected (b) mice. 4×10^5 lung cells were cultured for 72 hr. SI (stimulation index = c.p.m.$_{exp}$: c.p.m.$_{contr}$) is given in parenthesis. Standard errors (SE) did not exceed 10% for the PPD and 15% for the Con A responses.

RESULTS

Proliferative response of lung cells

Figure 1 shows the pattern of *in vitro* proliferation of lung cells. Neither the unfractionated population nor populations of lung cells depleted of plastic-adherent (PA) and/or NWA cells from healthy mice responded to PPD, but they showed a pronounced response to Con A. Surprisingly, proliferation of cells from infected lung in the presence of PPD was significantly decreased in comparison with non-stimulated (Group IV). The Con A-stimulated response was very low but was not inverted. Elimination of PA plus NWA cells result in restoration of the high response to Con A and in an appearance of the pronounced response to PPD (Group VI), whereas depletion of PA cells without additional cleaning led up to a partial restoration of the Con A response only, but had little effect on the PPD response (Group V). The population of cells that did not adhere to the plastic surface after 24 hr incubation contained up to 10% of cells with macrophage-like morphology, presumably incapable of lasting adhesion and, apparently, enriched for dendritic cells.[18] The removal of phagocytes loaded with carbonyl iron from this population resulted in full abrogation of suppression, comparable with that achieved after purification on nylon-wool column (Group VIII).

Our data suggest that the infected lung contains a population of functionally active T cells, some of which were sensitized against mycobacterial antigens in the course of infection. The proliferative response of these cells is, presumably, suppressed by PA, phagocytic and NWA cells. The appearance of the PPD-reactive cells in lung after contact with mycobacteria, and suppression of their PPD-specific response by PA and NWA lung cells, was further confirmed in the experiments with BCG-vaccinated mice (data not shown).

Suppression of proliferative response by lung cells

In order to confirm that the suppressor cells were indeed presented in lungs and to characterize the influence of infection, we studied the effect of lung cells on the proliferation of lymph node cells from mice immunized with FCA in co-culture experiments (Fig. 2). The pronounced antigen-specific response to PPD of immune lymph node cells was totally abolished in the presence of unfractionated lung cells from infected animals at the suppressor/responder ratio 1:4 and 1:8. The same doses of unfractionated cells from intact lungs had no effect (Fig. 2a). If PA cells were removed from the population of infected lung cells, the suppressive effect was still observed, whereas depletion of PA plus NWA cells fully restored the response (Fig. 2b). Pure plastic-adherent cells showed the most dramatic suppressive activity. PA cells from infected lungs abrogated the responses to PPD at the ratio 1:16 (Fig. 2c) and to Con A at the ratio 1:8 (Fig. 2d). PA cells from intact lung also inhibited the response of the PPD-immune lymphocytes, but the suppression was much less pronounced and required higher doses of lung cells (Fig. 2c). These data suggest that the development of tuberculosis infection highly increases the suppressive potency of adherent lung cells on proliferation of T lymphocytes.

Suppressor lung cells are macrophages

Since the abolition of suppressor activity in the plastic non-adherent lung cell population of infected mice required addi-

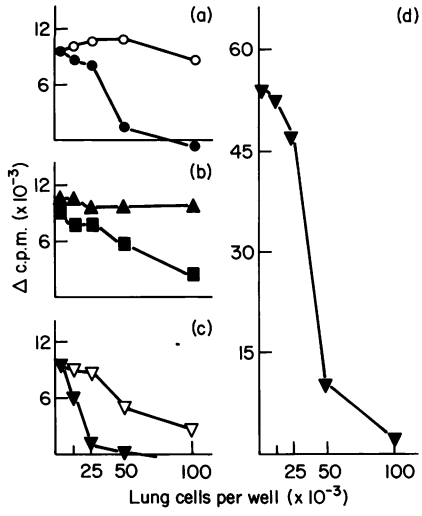

Figure 2. The proliferative response of FCA-immune lymph node cells is suppressed by the PA lung cells. 4×10^5 lymph node cells were co-cultured for 48 hr (Con A) or 72 hr (PPD) with indicated numbers of mitomycin-C-treated lung cells from infected (closed symbols) or intact (open symbols) mice in the presence of PPD (a, b, c,) or Con A (d). The response is expressed as Δ c.p.m. = c.p.m.$_{exp}$ − c.m.p.$_{contr.}$ (○) Unfractionated lung cells. (□) After PA cell removal; (△) after PA + NWA cells removal; (▽) pure PA cells. SE were less than 15%.

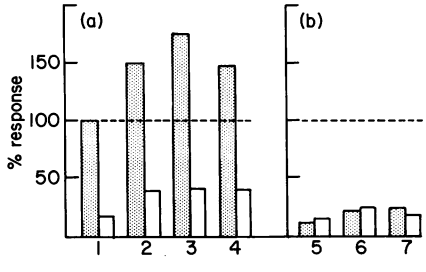

Figure 3. Lymphocytes from infected lungs are not involved in suppression of the proliferative response to PPD. (a) Enrichment for lung lymphocytes; (b) elimination of lung lymphocytes. 4×10^5 lymph node cells were cultured with (hatched bars) or without (open bars) PPD in the presence of 10^5 lung cells from infected mice: 1, control without lung cells; 2, high density fraction; 3, nylon-wool-purified; 4, nylon-wool-purified, PPD- and IL-2-prestimulated; 5, unfractionated; 6, Thy-1^+-depleted; 7, Ig^+-depleted.

tional purification on nylon wool (Figs 1 and 2), we studied whether B cells or a subset of NWA T cells could possibly participate in the development of suppression. The results of co-culture/cell-depletion experiments are shown in Fig. 3. One can see that co-culturing of immune lymph node cells with either: (i) high density ($\rho = 1{\cdot}065$–$1{\cdot}088$), lymphocyte lung cell fraction obtained from Percoll gradient, (ii) nylon-wool-purified cells from infected lungs or (iii) T cells stimulated *in vitro* with PPD and IL-2-containing supernatant resulted in stimulation rather than suppression of the response (Fig. 3a). On the other hand, it was shown that the suppressive activity of lung cells was not altered after removal of either Thy-1.2^+ cells by treatment with antiserum and complement or Ig^+ cells by panning on the anti-Ig antibody-coated plastic (Fig. 3b).

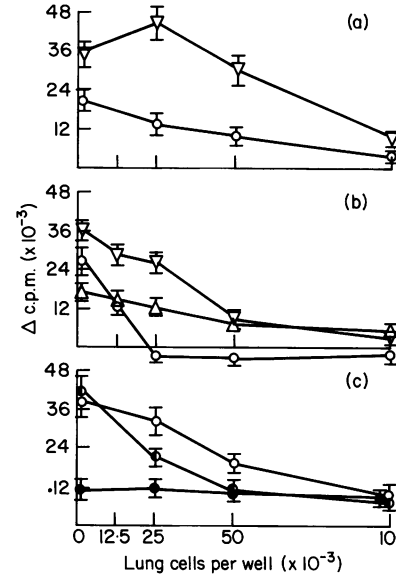

Figure 4. Suppression of proliferative responses of lymph node cells to mycobacterial and irrelevant antigens by unfractionated (a), PA (b) lung cells and involvement of responder lymphocyte populations in suppression of the anti-PPD response (c). Lymph node cells were taken from mice immunized with FCA (○); *S. aureus* (▽) and OVA (△) and cultured with indicated numbers of lung cells for 72 hr. (○) Untreated PPD-reactive lymphocytes; (◐) treated with anti-Lyt-2 antibodies; (●) treated with anti-I-J^k antiserum.

Taken together, the data from Sections 1–3 suggest that PA, NWA, phagocytic cells, i.e. the cells with characteristics of macrophages, represent the main suppressor cell population which resides within the lung tissue and undergoes considerable activation in the course of infection.

Possible specificity of suppression and involvement of a responder cell population

The observations stimulated a more detailed study of possible involvement of some infection-specific mechanism(s) in the suppression of proliferative responses. Firstly, the response to mycobacterial antigen PPD in the presence of high doses of suppressor lung cells from infected animals showed an inverted pattern, that is it was lower in PPD-stimulated cultures than in controls. Secondly, the PPD-stimulated response of immune lymph node cells was totally abolished in the presence of PA infected lung cells at a ratio of 1:10 (Fig. 2c), whereas the polyclonal Con A-induced response was resistant to the action of PA suppressor cells added at this low dose (Fig. 2d). Thus, we further studied the possible specificity of suppression by comparing the inhibition of immune lymphocyte responses against PPD and non-mycobacterial antigens (Fig. 4).

The unfractionated cells from infected lungs significantly suppressed the proliferation to PPD of FCA-immune lymphocytes when added at ratios of 1:8 and 1:16, but the response to *S. aureus* antigen was inhibited by a high dose (1:4) of these cells only (Fig. 4a). This observation was confirmed by even more revealing results with the pure PA lung cells (Fig. 4b). Since the level of the *S. aureus*-specific response was significantly higher than that of the PPD-specific response, the result obtained could possibly be explained by differences in the strength of activating

Table 1. The presence of NWA cells in the responder population is required for suppression*

Exp.	PA lung cells	PPD	C.p.m. (10^{-3})	SI	Suppression (%)
(a)	—	+	31·2	7·8	
		−	4·0		
	4×10^3	+	9·8	4·3	69
		−	2·3		
	4×10^3	+	5·8	1·6	81
		−	3·7		
(b)	—	+	6·1	6·8	
		−	0·9		
	4×10^3	+	6·0	7·5	2
		−	0·8		
	40×10^3	+	5·0	5·6	18
		−	0·9		

* 4×10^5 FCA-immune unfractionated (a) or nylon wool-purified lymph node cells were cultured for 96 hr with or without PPD in the absence or presence of PA cells from infected lungs.

SI, stimulation index.

signals. Nevertheless, the weak but significant response to OVA was also resistant to the suppressive action of low doses of PA-infected lung cells (Fig. 4b). These results suggest that the antigen-specific suppressor mechanism is activated in lungs in the course of tuberculosis along with the non-specific suppression, and raises the question of the involvement of cells carrying antigen-specific receptors. Since the removal of the Thy-$^+$ or the Ig$^+$ cells from the lung cell population did not alter the suppression (see above), we turned to a responder lymph node cellular population.

Figure 4c shows that the cytotoxic treatment of the responder population with mAb against the Lyt-2 product, a common marker of T effector (CTL and Tse) class I-restricted lymphocytes, had no effect on either the ability of the remaining cell population to respond to PPD, or to be suppressed by lung cells. In contrast, treatment with the antiserum against the I-J determinant, a marker of receptor–anti-receptor Ia-restricted interactions in suppressor circuits,[19] led to a decrease of total proliferative capacity of the lymph node cells, but resulted in a loss of suppression of the PPD-specific response by lung cells (Fig. 4c).

Very similar results were obtained when we used a nylon-wool-purified lymph node cell population as responders. Again, proliferation was weak (Table 1b) in comparison with control cultures (Table 1a), but even pure PA lung cells could not suppress the response of nylon-wool-purified lymph node cells any longer.

A low level of proliferation itself should not be considered as a reason for a failure of the lung cells to suppress the antigen-specific response. Indeed, the addition of the anti-I-A^k 10-2-16 mAb to cultures led to an eightfold decrease of the PPD-specific response, but still left a low but significant I-E-restricted[20] response untouched; the latter was effectively suppressed by lung cells (data not shown).

Our results suggest that the induction of the PPD-specific interactions in the responder population by the lung mycobacteria-sensitized macrophages, which depend on the presence of I-J-positive and/or NWA cells, could be one of the possible causes of the infection-specific suppression of proliferative responses (see the Discussion).

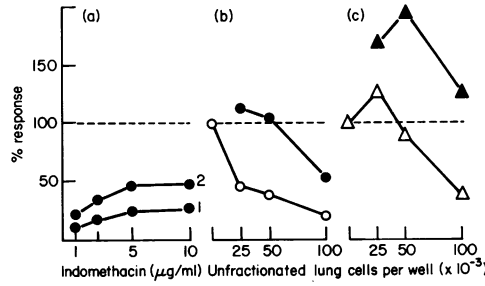

Figure 5. Indomethacin influences differently infection-specific and non-specific suppression. 100% response proliferation of FCA-immune (circles) or *S. aureus*-immune (triangles) lymph node cells in indomethacin- and lung cell-free cultures. (Closed symbols) indomethacin added. (a) 50×10^3 (curve 1) or 25×10^3 (curve 2) PA lung cells per well; (b and c) 10 μg/ml indomethacin, unfractationed lung cells.

Non-specific suppression is caused by prostaglandins

It is well known that the suppressor activity of monocytes and macrophages in several experimental systems depends on PGE production, which inhibits proliferation of T cells by blockade of IL-2 secretion.[21] Thus, we studied the suppression of the PPD-specific and the *S. aureus*-specific proliferation of cells from infected lungs in the presence of indomethacin, a drug which causes an inhibition of PGE synthesis.[22]

As shown in Fig. 5a, indomethacin in a wide spectrum of concentrations influenced the suppression of the PPD-specific response by the pure PA lung cells only weakly. When the unfractionated lung cell population with a less pronounced suppressive activity was used as a source of suppressors cells (Fig. 5b), the effect of indomethacin was significant at the low concentrations of lung cells but again was weak in the presence of 10^5 lung cells per well. In contrast, the suppression of *S. aureus*-specific response by the high dose of unfractionated lung cells was fully corrected by indomethacin (Fig. 5c), suggesting that the PGE production could be a major mechanism of non-specific suppression. An increment of proliferative activity in *S. aureus*-specific indomethacin-treated cultures was, presumably, a consequence of inhibition of PGE production by the lymph node macrophages as well.

Taken together these results indicate that the dual suppressive mechanism is possibly activated in the course of infection: non-specific suppression is mediated mainly by PGE, while the suppression of response to mycobacterial antigens could not be explained solely by PGE synthesis.

DISCUSSION

The purpose of the present study was to define a possible involvement of the interstitial lung cells in the regulation of immune responses in mice, and to investigate the influence of tuberculosis infection on the development of suppression of the proliferative response. In agreement with the data reported by P. Holt and his colleagues for rat and human experimental systems,[4–6] it was shown that the murine interstitial lung cells

bearing characteristics of macrophages behaved as suppressors of lymphocyte proliferation. The progression of the infectious process strongly augmented the suppressive activity of lung macrophages (Figs 1 and 2). It was found earlier that the infectious agents or their products are potent activators of macrophage-mediated suppression. Thus, suppressor activity has been reported for splenic macrophages of rats injected with group A streptococcal cell walls,[23] for macrophages from liver granulomas of *Schistosoma mansoni*-infected mice,[24] and for aqueous extracts of pulmonary granulomas from BCG-immunized high responder mice.[25] In the work of Mbawuike and Herscowitz[11] it was shown that the suppressor potency of peritoneal macrophages was much higher if the hosts had been pretreated with killed BCG. Our results confirm the strong augmentation of suppression after infection of host macrophages by the intracellular infectious agent.

The exact mechanisms of macrophage-mediated non-specific suppression have not been characterized completely as yet. Several recent studies proposed the role of non-specific cytokines, but it seems likely that the nature of these factors depended mainly on the test system and on the source of suppressor macrophages: MIF and IL-1;[26,27] TGF-β, but not PGE_2;[23] IFN-α/β and PGE_2;[24] PGE_2 and IL-2 expression-inhibition factor;[28] superoxide anion and PGE_2;[11] IL-1 and TNF;[29] and so on. Our data suggest that non-specific suppression mediated by the interstitial lung macrophages depends strongly on PGE production by these cells, since the response to *S. aureus* antigen was restored completely in the presence of indomethacin. Nevertheless, inhibition of PGE synthesis only slightly influenced the suppression of the PPD-specific response (Fig. 5).

The most intriguing question is the nature of macrophage-mediated infection-specific suppression, which is induced simultaneously with the PGE-mediated non-specific suppression. The fact that the adherent mononuclear cells from infected donors participate in suppression of responses, specific to the antigens of the causative agent, is well documented for several bacterial and parasitic diseases.[30-32] Since there is no definite evidence that macrophages are able to synthesize antigen-specific receptors, possible interactions of suppressor macrophages with lymphocytes were studied (refs 33 and 34 and many others). It was found[15] that, in addition to several non-specific mechanisms, PPD-specific suppression in patients with tuberculosis was mediated by $CD8^-$, $Fc\gamma R^+$ lymphocytes, probably interacting with monocytes.

In this study it was shown that, although lung- wall lymphocytes were not involved in the suppressor pathway (Fig. 3), the PPD-specific suppression developed fully only in the presence of I-J-positive and/or NWA cells in the responder lymph node cell population, but required no Lyt-2^+ lymph node lymphocytes (Table 1). These data are in agreement with the finding of Ferrick & Herscowitz (10), who reported that the interaction between the BAL macrophages and I-J^+ Lyt-2^- L3T4^+ T cells is necessary for generation of suppression of antibody response against sheep red blood cells (SRBC). It should be emphasized that non-specificity of suppression in the work of Ferrick & Herscowitz could well be only apparent because all types of interacting cells were co-cultured in the presence of the antigen for 4 days before testing. It is also of interest that the response against SRBC was also controlled by 'usual' Lyt-2^+ Ts effector cells: elimination of the latter elevated the response but did not alter macrophage-mediated suppression. In our experiments the depletion of Lyt-2^+ T cells from the responder population resulted in a response of the *remaining* L3T4^+ lymphocytes *equal* to that of the *whole* unfractionated population (Table 1).

Dependence upon the I-J/class II-restricted interactions is a well-known feature of antigen-specific suppressor pathways (reviewed in ref. 19). The requirement for cells, proposed by our results, could probably be explained either by the involvement of signal-transducing (TsF-presenting?) cells from the macrophage lineage in the I-J/I-E-restricted suppressor cascade,[35] or by the adherence of Ts to the nylon wool.

However, it cannot be ruled out that some other mediators besides PGE (the most likely candidates are T-cell growth factor and IL-1; see above) provided suppression in our experimental system, and that the secretion of the latter was induced in the presence of PPD but not in the presence of the irrelevant antigens used. This supposition is enough to explain the differences in indomethacin action on PPD-specified and *S. aureus*-specific responses (Fig. 5).

Still another possibility is that the suppressor macrophages (up to 10% of which contain mycobacteria; our unpublished observations) interact with immune T cells but, in the presence of soluble mycobacterial antigens, fail to produce a second positive signal for proliferation, or produce a negative signal.[36]

ACKNOWLEDGMENT

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