9. J. Bloland, J. Troguet, and S. J. Blen, Cardiovasc. Res., 10, 474 (1976).
10. C. T. Jones and J. W. Ritchie, J. Physiol. (London), 285, 381 (1978).
11. M. Karnovsky and L. Roots, J. Histochem. Cytochem., 12, 219 (1964).
12. T. N. James, in: Sudden Death [Russian translation], Moscow (1982), p. 151.

INDUCTION OF BRONCHOSPASM BY MEDIATORS OF ANTIGEN-SPECIFICALLY STIMULATED LYMPHOCYTES

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Soluble mediators of cellular immunity, namely lymphokines (LK), bring about interaction between the various types of cells that participate in the immune response [3]. The number of LK described in the literature [6] is 56. The target of their action may also be cells not directly concerned with the immune response (fibroblasts, epithelial cells, platelets, erythrocytes, etc.).

No data could be found in the literature on the effect of LK on smooth muscle tissue function. Yet this problem is of both scientific and practical importance in the elucidation of the pathogenesis of the infectious-allergic form of bronchial asthma and of the asthmatic phenomena associated with respiratory infections on a wider plane. This disease is one of the most important respiratory diseases and it is usually associated with delayed-type hypersensitivity (DTH) to microbial antigens [1, 2].

This paper describes the results of a study of the action of antigen-specific and antigen-nonspecific LK on the bronchial smooth muscle of isolated guinea pig lungs.

EXPERIMENTAL METHOD

Experiments were carried out on 110 noninbred guinea pigs, in 20 of which DTH was induced against brucellas of the vaccine strain VA-19, and in another 20 against *Staphylococcus aureus* strain Cowan 1. The methods of sensitization were described previously [2]. After 1-1.5 months the development of DTH was investigated by skin tests and the blood leukocyte migration inhibition test (LMIT), and only animals giving a positive reaction were used in the subsequent experiments. All experiments on the animals were performed under open ether anesthesia. Total LK were obtained as the 24-h supernatant of lymphocytes in conical test tubes by the method in [7]. The thymus, lymph nodes, and spleen were homogenzied and a suspension containing 5×10^7 cells/ml was cultured in medium 199 with the addition of 100 μg/ml of staphylococcal or 200 mg/ml of brucella antigen (AG) (the AG were obtained by ultrasonic disintegration of the microorganisms [2]), 10% bovine serum, and 1000 I.U. each of penicillin and streptomycin. The presence of LK in the supernatant was tested by the peripheral blood LMIT in intact animals by a modified method [4]. Supernatants active in the indirect LMIT were used for investigation of the reactions of isolated guinea pig lungs (IL). Parallel with these experiments the following controls were used: 1) control of the medium for incubation of lymphocytes (MC); 2) AG in a working concentration (AGC); 3) control of supernatants without AG (LKC) and of the supernatant after incubation of lymphocytes with heterologous AG (HAGLK), at which an ultrasonic extract of *Escherichia coli* in a dose of 200 μg/ml was used. The test agents were injected into the IL perfusion flow in a volume of 3 ml.

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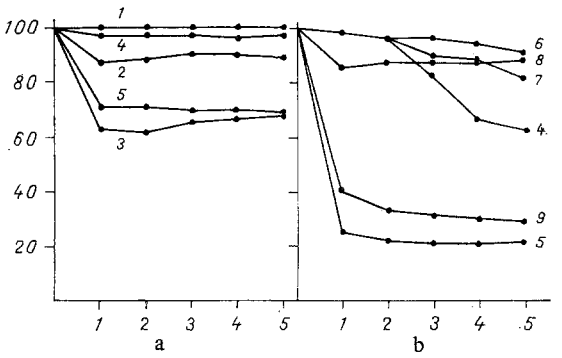

Fig. 1. Responses of isolated lungs to lymphocyte mediators. a) LK obtained from animals sensitized with brucellas; b) LK obtained from animals sensitized with staphylococci. Abscissa, time after injection of test agent into flow of perfusion fluid (in min); ordinate, amplitude of respiration of IL (in % of initial level).

Investigation of IL was carried out in a modification of the method. Respiration of IL was recorded by means of an original temperature transducer and electroencephalograph. The vascular system of IL was perfused with Tyrode solution made up in dextran with the addition of 2% gelatin. The perfusion fluid was tested for the presence of histamine by a biological method [5]. The patency of the bronchi was judged from the decrease in amplitude of respiration of IL during the 5 min after injection of the test agent. Histological and morphological studies of sections of IL were carried out 2 min after exposure to the agent.

EXPERIMENTAL RESULTS

In the experiments of sieres I LK specific against brucellas were used. The results are summarized in Fig. 1a.

It will be clear from Fig. 1 that after injection of LK into IL of animals with DTH to brucellas the patency of the bronchi was reduced by the end of the first minute by almost 40% compared with initially. Later the amplitude of respiration changed only a little and remained low throughout the period of observation. LKC and AGC had little or no effect on bronchial patency. Differences between responses in the experiment and control were significant ($P < 0.05$).

Similar results were obtained on testing the action of LK on IL of intact animals (Fig. 1a). Responses of IL of sensitized and intact animals did not differ significantly ($P > 0.05$).

In the experiments of series II the action of LK obtained from lymphoid organs of guinea pigs sensitized with staphylococci on IL of intact animals was investigated (Fig. 1b and 2). Injection of LK in all experiments evoked a bronchoconstrictor effect: the level of bronchial patency by the end of the first minute had fallen on average by 75% (Fig. 1b) and responses of complete bronchospasm were observed (Fig. 2a). In the controls at this period there was no response, but in some experiments after the end-3rd minute or later there was a gradual fall in the amplitude of respiration (compare Figs. 1b and 2c, d). The differences between responses to LK and the control preparations were highly significant ($P < 0.01$).

Histological study of the IL sections showed that 2 min after injection of LK irregular spasm of the smooth muscle of the bronchioles was present, accompanied by marked disturbances of staining properties, intercellular edema, and separation into layers (Fig. 3). Edema fluid accumulated beneath the epithelium of the bronchioles and bronchi, especially in the submucosal layer, where there were accumulations of lymphocytes and macrophages. The bronchial epithelium exhibited moderate edema only of the basil portions. Around the bronchioles the alveoli were grossly distended and the respiratory passages dilated. These changes were not present in control preparations.

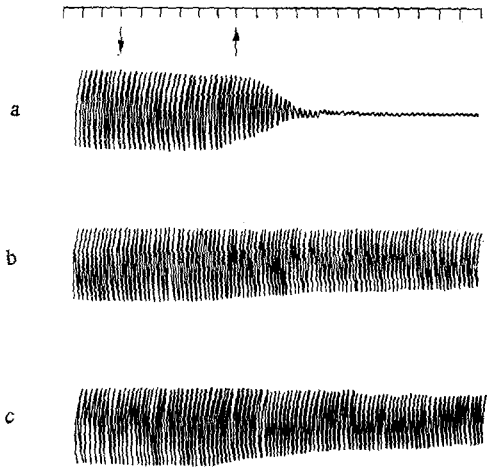

Fig. 2. Pneumogram of isolated lungs under the influence of LK specific for staphylococci. a) Action of LK, b) action of LKC; c) action of HAGLK. Time marker (10 sec) above pneumograms. Arrows indicate time of injection of agent.

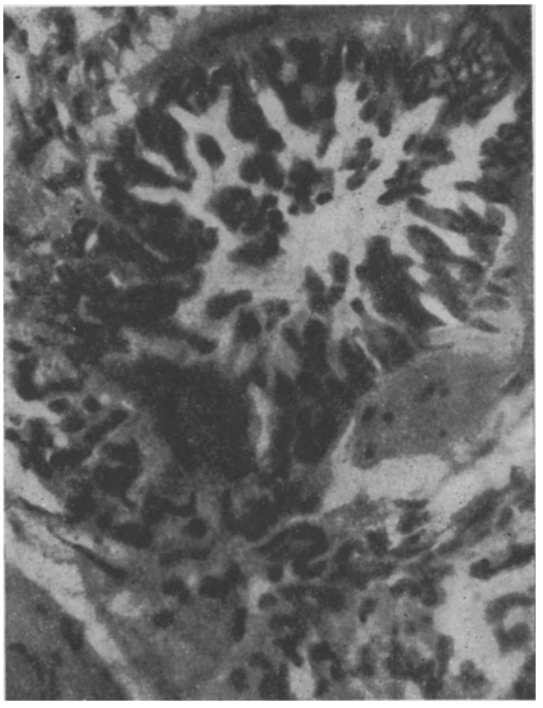

Fig. 3. Irregular contraction of bronchial musculature edema. Hematoxylin-eosin, 200×.

Analysis of perfusion fluid flowing from IL showed absence of histamine and other spasmogenic agents in all the series of experiments with LK.

Dialysis of LK obtained from animals immunized with staphylococci against lymphocyte incubation medium or physiological saline for 48 h did not change the spasmogenic properties of LK and the dialysate did not acquire spasmogenic activity. Heating LK for 30 min at 56°C almost completely eliminated the spasmogenic factor.

To compare the bronchospastic activity of the test factor with that of the already well studied LK which inhibits cell migrations the values of the LMIT index of intact leukocytes and those of the spastic response (expressed as the reduction in amplitude of respiration in

the first minute, in %) of intact IL under the influence of the same batch of supernatant, were subjected to correlation analysis. In both the 1st and the 2nd group of experiments no significant correlation was found between these parameters. The spasmogenic properties of LK were thus evidently unconnected with the presence of leukocyte migration inhibiting factor.

As the result of these investigations the spastic action of a substance secreted by antigen-specifically stimulated lymphocytes on the smooth muscle of the bronchi was revealed for the first time. The spasmogenic activity of lymphocyte supernatants could not have been due to the possible presence of immune antigen–antibody complexes in them not only because of the short period of culture, but also because of the absence of complement in the system and of histamine in the perfusion fluid, for complement and histamine are known to be essential components of the mechanism of aggressive action of immune complexes on smooth muscle. An Ig–E-mediated mechanism was completely ruled out by the character of the well studied model of DTH [2].

It is difficult on the basis of these experiments to give a precise description of the characteristics of the spasmogenic factor specifically secreted by lymphocytes; further research in this direction is in progress. However, the substance was shown not to dialyze, to be thermolabile, and not to be connected with activity of the LK which inhibits cell migration. Like many of the biological properties of most of the known LK which have already been studied, the action of the spasmogenic factor is manifested on both sensitized and intact smooth-muscle tissue.

The absence of histamine or of other spasmogenic substances in the IL perfusion fluids is evidence that chemical mediators of constrictor reactions of smooth-muscle tissue of this kind do not participate in bronchospasm, and the possibility cannot be ruled out that LK has a direct action on smooth muscle.

The spasmogenic effect of LK has thus been established in allergy of delayed type to microbial antigens and has been demonstrated with equal constancy on two models of sensitization with unrelated microorganisms; this can evidently be taken to reflect the universality of this phenomenon in the field of microbial sensitization with a course resembling that of delayed allergy.

LITERATURE CITED

1. A. D. Ado, Special Allergology [in Russian], Moscow (1976).
2. N. D. Beklemishev and G. S. Sukhodoeva, Allergy to Microorganisms in Clinical and Experimental Practice [in Russian], Moscow (1979).
3. N. V. Medunitsyn, V. I. Litvinov, and A. M. Moroz, Mediators of Cellular Immunity and Intercellular Interaction [in Russian], Moscow (1980).
4. D. K. Novikov and V. I. Novikova, Cellular Methods of Immunodiagnosis [in Russian], Minsk (1979).
5. V. I. Uspenskii, Histamine [in Russian], Moscow (1963).
6. B. R. Bloom, J. Immunol., 124, 2527 (1980).
7. J. L. McCoy, J. A. Braatz, et al., J. Immunol., 124, 2786 (1980).