EXPERIMENTAL STUDY OF HYPERSENSITIVITY OF DELAYED TYPE AND IMMUNITY AGAINST TUBERCULOSIS

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Hypersensitivity of delayed type was studied by means of immunospecific reactions of blast transformation and inhibition of migration in experiments on 40 guinea-pigs infected with strain $H_{37}Rv$ or vaccinated with BCG. The highest degree of blast transformation of sensitized leukocytes under the influence of PPD occurred while the intensity of immunity was high (2 months after vaccination), whereas during widespread tuberculosis the number of blast cells in the culture was low and many cultures were destroyed. Maximum inhibition of migration of sensitized leukocytes was observed in widespread tuberculosis (2.5 months after infection), whereas in the case of high immunity inhibition of migration was less marked.

Immunospecific interaction of tuberculin and mycobacterial antigens with sensitized cells in vitro has been used for many years as a model for the study of allergy and immunity in tuberculosis [5, 11, 13, 15, 17]. In the last decade, several highly sensitive methods have been developed for studying hypersensitivity of delayed type in vitro (the blast transformation reaction, inhibition of migration of macrophages, cytotoxic effect of lymphocytes), and these have revealed new general immunological principles; moreover, many of these investigations have been undertaken with mycobacteria and their antigens [6-8, 10, 12, 14, 16].

The study of the correlation and connection between hypersensitivity of delayed type and immunity against tuberculosis is a basic problem in the immunology of tuberculosis. Many investigations have been devoted to this problem, and the subject has been surveyed most fully by Arnason and Waksman [4]. However, it is still not absolutely clear whether hypersensitivity of delayed type is one of the mechanisms of defense against tuberculosis, nor is it known whether hypersensitivity always accompanies the state of immunity.

The object of the investigation described below was to use reactions in which the principal active cells are lymphocytes sensitized to mycobacterial antigens, in order to study the correlation between hypersensitivity of delayed type and immunity in tuberculosis.

EXPERIMENTAL METHOD

The blast transformation test was carried out by the method described by Pearmainet al. [12], Ling [10], and others. It is based on the transformation of small lymphocytes into blast cells under the influence of antigen with which the cells or their precursors have been in contact in the past.

Circulating blood leukocytes obtained from guinea-pigs by allowing the blood to stand with 10% gelatin were suspended in autologous plasma, diluted with Eagle – MEM medium (to a concentration of $2 \cdot 10^6$ cells/ml with 20% plasma, in a total volume of 5 ml) and cultivated in 50-ml flasks in an incubator at 37°C

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TABLE 1. Blast Transformation of Lymphocytes of Guinea-Pigs Infected with Strain $H_{37}Rv$ or Vaccinated with BCG, under the Influence of PPD (30 μg/ml)

Index	2 months after vaccination with BCG	2.5 months after infection with $H_{37}Rv$	Control
Number of animals	10	10	10
Mean percent of blast cells:			
with PPD	3.83*	0.72	0.95
without PPD	0.86	0.54	1.12

* $P < 0.001$ (relative to control group).

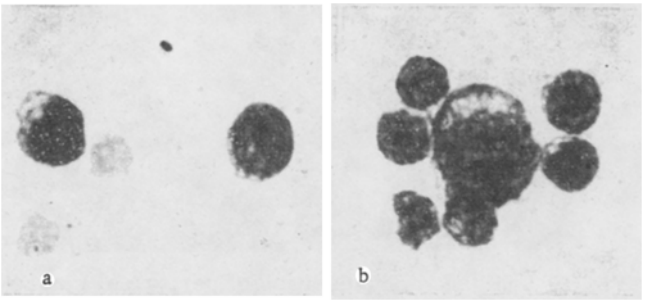

Fig. 1. Three-day cultures of circulating guinea-pig lymphocytes with PPD (30 μg): a) stimulated culture of control guinea-pig; b) stimulated culture of vaccinated guinea-pig (2 months after vaccination). Romanovsky–Giemsa stain, 900 ×.

with (experiment) or without (control) 30 μg PPD/ml. After 3 days the culture fluid was collected and centrifuged at 1000 rpm, after which films were made from the cell residue, fixed, and stained by the Romanovsky–Giemsa method. In these films 2000 lymphocytes and blast cells were counted and the number of blast cells expressed as a percentage.

The inhibition of migration test was carried out by the method of George and Vaughan [7] in David's modification [7]. It consists essentially of the inhibition of migration of sensitized or normal macrophages by the migration suppressing factor secreted from lymphocytes on their contact with antigen [6].

Cells of the peritoneal exudate taken 3 days after injection of thioglycol broth were sedimented by centrifugation at 1000 rpm, washed twice with medium No. 199, then collected in capillary tubes 1 mm in diameter, again centrifuged at 500 rpm, and placed in transparent plastic chambers containing medium No. 199 with 20% homologous serum and with (experiment) or without (control) 200 μg/ml PPD. The area of migration of the cells was projected on photographic film after 48 h, and the pieces of film were cut out and weighed. The migration index (J) was calculated by the formula:

$$J = {}^{Pe}/_{P_c},$$

where P_e is the mean weight of the samples of migration area in the experimental series and P_c the same in the control.

The indices listed above were studied in animals with a high intensity of immunity (2 months after vaccination with 1 mg BCG [1]) and in animals with widespread tuberculosis (2.5 months after infection).

EXPERIMENTAL RESULTS

In the case of animals with a high intensity of immunity (2 months after vaccination), stimulated three-day cultures of circulating blood cells of the guinea-pigs were found (Table 1; Fig. 1) to contain 3-6% of

TABLE 2. Inhibition of Migration of Leukocytes from Guinea-Pigs Infected with Strain $H_{37}Rv$ or Vaccinated with BCG, under the Influence of PPD (200 μg/ml)

Index	2 months after vaccination with BCG	2.5 months after infection with $H_{37}Rv$	Control
Number of animals	10	10	10
Mean percent of blast cells	0.72*	0.34	1.05

* $P < 0.001$ (relative to control group).

Fig. 2. Inhibition of migration of peritoneal macrophages of a guinea-pig infected with strain $H_{37}Rv$ (2.5 months after infection); a) chamber without PPD (control); b) chamber with 200 μg PPD.

blast cells, whereas 2.5 months after infection the number of blast cells was much smaller and many of the cultures were destroyed (from six of the ten guinea-pigs). Cultures without PPD (reaction control) were less frequently destroyed, as also were those in the group with widespread tuberculosis.

A study of the inhibition of migration (Table 2) of sensitized peritoneal exudate cells (under the influence of PPD) showed that 2.5 months after infection there was marked inhibition of migration, while at the height of the immune response after PCG vaccination, inhibition of migration was much weaker (Fig. 2).

Comparison of the results of these tests thus showed that if marked immunity was present, blast-formation was stimulated in the lymphocyte cultures to the greatest degree, whereas lymphocytes of animals with widespread tuberculosis frequently not only were not stimulated by PPD but, on the contrary, were destroyed. On the other hand, whereas inhibition of migration was slight when immunity was at a high level, in the case of widespread tuberculosis the inhibition of migration was always well marked. Possibly despite the fact that both these phenomena are related to hypersensitivity of the delayed type, two different populations of lymphocytes are responsible for them: some are stimulated and transformed under the influence of the antigen against which they are immune, while others are injured by the antigen to which they are sensitized, or they interact with it with the liberation of a factor inhibiting migration, so that migration of macrophages is subsequently suppressed. The possibility is not ruled out that the same cells, in different states of functional activity, may take part in the two tests described above.

A number of investigations [2, 3, 9, 10] have indicated the existence of several lymphocyte populations, but it is not yet known how the properties of the different populations of small lymphocytes correlate with their ability to react with antigen in vitro. Very probably different populations of lymphocytes react with mycobacterial antigen in vivo also, in some cases perhaps through a mechanism of immunity, and in others through hypersensitization.

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