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PASSIVELY TRANSFERRED CELLS LABELLED WITH H³ THYMIDINE IN DELAYED TYPE HYPERSENSITIVITY REACTION IN THE GUINEA PIG LUNG

By

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The role of mononuclear cells in delayed type hypersensitivity reactions is still uncertain and a study of the behaviour of passively transferred, sensitized lymphocytes in delayed type hypersensitivity reactions may provide information of interest regarding this role.

In 1961, Najarian & Feldman (5) reported that when tritiated-thymidine-labelled lymphoid cells obtained from BCG sensitized guinea pigs were passively transferred into homologous guinea pigs, specific attraction of labelled cells was noted at the sites of PPD injections 24 hours later. Labelled non-sensitized lymphoid cells did not appear at PPD injection sites, nor did labelled sensitized cells accumulate in non-specific inflammatory lesions. On the other hand, other investigators reported that there was no significant increase in the number of transferred cells at the skin test sites (1, 2, 7, 8). Najarian & Feldman repeated their studies and still found a somewhat higher level of labelled cells in sensitized donors at specific skin test sites than at control sites (6). These studies were carried out using the skin as the site of the delayed type hypersensitivity reaction.

Currently, the lung provides a good site for the delayed type hypersensitivity reaction in actively as well as passively

sensitized animals (3, 4). In addition, the lung appears in some respects superior to the skin, since passively transferred cells circulate through the lung and have more opportunity to contact the inhaled antigen. Consequently, the lung was used in current studies to examine the behaviour of transferred sensitized lymphoid cells in the passively induced delayed hypersensitivity reaction in guinea pigs.

MATERIALS AND METHODS

For active immunization, guinea pigs were given a single intramuscular injection of 0.5 ml of paraffin oil containing 0.5 mg of dry, killed tubercle bacilli. Four to eight weeks later, the animals were tested for tuberculin hypersensitivity by injection of 0.1 μ g of purified protein derivative (PPD) intradermally into the shaved dorsal skin. Animals with a strong positive reaction received intravenous injections of 0.5 mg of dry, living, bovine tubercle bacilli suspended in saline solution. Starting on the same day, each animal received 500 μ c of tritiated thymidine injected intraperitoneally daily for 2 days and then 800 μ c twice daily for the following 2 days.

On the 5th day after intravenous injection of tubercle bacilli and 8 hours after the last thymidine injection, the spleens were excised and cut into small pieces in medium 199 and sieved through a 200-mesh stainless steel screen. The isolated cells were washed with chilled medium 199 and resuspended in the medium. Smears of the sediment were made for differential analysis and autoradiography. Total cell numbers were determined in a hemocytometer, and cell viability was measured by the Eosino Y exclusion test. In this study cell viability was over 90 per cent. An average of 19-27 per cent of the mononuclear cells in the suspension was labelled. The number of grains overlying individual cell nuclei was quite variable, indicating either concentration of the isotope as a result of mitoses during the 4-day labelling procedure or dilution as a result of mitoses after cessation of labelling.

To each recipient, 4×10^5 cells were transferred intravenously. Immediately thereafter, 10 ml of 0.2 mg of PPD per ml were aerosolized through a Vaponephrine nebulizer into the chamber containing guinea pigs. Uniform distribution of the aerosol was ascertained with a small fan in the chamber. Four animals were sacrificed 3, 6, 24 and 48 hours after aerosol exposure. Lungs were excised and radioautography was performed by the dipping method. Immediately after developing and acid fixing, tissues were stained with hematoxylin eosin. The number of cells labelled with tritiated thymidine was counted and expressed as a percentage of all cells.

The first control consisted of normal guinea pigs, sacrificed 3, 6, 24 and 48 hours after intravenous injection of labelled sensitized cells without exposure

to PPD, and treated as stated above. The second control group consisted of normal guinea pigs, injected with a mixed suspension of lymphoid cells derived from non-labelled sensitized animals and from labelled non-sensitized animals. The guinea pigs were then exposed to PPD. Non-sensitized labelled lymphoid cells were obtained from the spleens of guinea pigs which were injected with tritiated thymidine in an identical manner to the sensitized guinea pigs. Non-sensitized labelled cells comprised 5 to 15 per cent of the mononuclear cells. In the third control group, the guinea pigs received labelled sensitized cells and were exposed to 500 ppm of SO_2 for 20 minutes. The guinea pigs in the fourth control group were transfused with tritiated non-sensitized cells and were exposed to PPD inhalation. Each control consisted of 3 guinea pigs. The number of tritiated cells was compared in comparable groups by chi square test.

RESULTS

Microscopic examination of lungs 3 to 6 hours after exposure revealed a predominant infiltration by polymorphonuclear cells with much less penetration of mononuclear cells into the septa in passively sensitized guinea pigs, but the number of mononuclear cells gradually increased. After 20 hours, when the reaction was most striking, there were large collections of mononuclear cells, mostly lymphocytes in the peribronchial and perivascular areas and in the alveolar septa. Guinea pigs exposed to SO_2 showed quite similar reactions in the lung and, after 24 hours, mononuclear cells were quite predominant in the lung. Non-sensitized guinea pigs showed no obvious histological changes following PPD inhalation.

The most important finding was the presence of the labelled lymphoid cells in the reaction site. The number of cells with incorporated tritiated thymidine is shown in Table 1. There was no difference between specimens taken after 3, 24, and 48 hours. Six hours after exposure, the percentage of cells with incorporated tritiated thymidine was somewhat higher in experimental guinea pigs than in the controls, although the difference was not statistically significant in comparison with two other comparable groups ($0.2 > P > 0.1$).

The percentage of labelled cells obtained from normal guinea pigs was less than that of cells from sensitized guinea pigs after injection of tritiated thymidine. Accordingly, the

TABLE 1
Percentage of Labelled Transferred Cells in the Lung.

Intravenous injection	Inhalation	After inhalation							
		3 hrs.		6 hrs.		24 hrs.		48 hrs.	
		Reaction	Labelled cells	Reaction	Labelled cells	Reaction	Labelled cells	Reaction	Labelled cells
Labelled sensitized spleen cells	None	— ±	30 3964	—	30 4464	—	34 3673	—	7 2034
	PPD	± +	54 6830	+ + +	52 5478	+ + +	87 7390	+ + +	6 2875
	SO ₂	+	23 3345	+ + +	33 4210	+ + +	42 4410	+ + +	8 2621
Labelled non-sensitized spleen cells	None	—	16 3471	—	16 3628	—	14 3240	—	—
	PPD	— ±	13 3221	—	17 3532	—	16 3030	—	—
	SO ₂	+	16 3354	+ + +	22 4320	+ + +	15 3378	—	—
Labelled non-sensitized spleen cells + Non-labelled	None	—	13 4012	—	12 3216	—	13 3218	—	—
	PPD	+	16 4354	+ +	18 3864	+ +	18 3479	—	—

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number of labelled cells in the lungs of guinea pigs after injection of labelled non-sensitized cells was less than that of guinea pigs injected with labelled sensitized cells, but significant numbers of labelled cells were observed in the lungs. Non-labelled sensitized spleen cells and labelled non-sensitized cells were intravenously injected into two groups of guinea pigs. One group was challenged by PPD inhalation and one group was not. There was no marked difference in the number of labelled cells in the lungs of these two groups.

DISCUSSION

There is no doubt that transferred lymphocytes play a significant role in the passively transferred delayed type hypersensitivity reaction. Transferred lymphocytes are thought to be attracted by antigen, to react with it, and to release a migration inhibitory factor, resulting in stasis of other cells around it. Therefore it was thought that the number of the transferred lymphocytes at the reaction site should be much higher in experimental animals than in controls. Najarian & Feldman (5) reported that the percentage of labelled cells was up to 12 per cent of mononuclear cells, which was significantly higher than the control. On the other hand, others found no significant increase of transferred labelled cells at the reaction site (1, 2, 7, 8). Their studies were carried out using the skin as the site of the delayed type hypersensitivity reaction.

Since passively transferred cells enter the pulmonary circulation, there is a much greater opportunity for the cells to get into contact with antigen in the lung than the skin. Consequently, the lung was thought to be the ideal site for this reaction and was therefore used in this experiment. No significant difference of labelled transferred cells was found in this study in comparison with controls, except 6 hours after the initiation of delayed type hypersensitivity reaction, and this difference was not statistically significant. This does not exclude the role of transferred cells at the site of antigen exposure, since only 19 to 27 per cent of transferred monocytes

were labelled and since the labelled cells do not necessarily represent the immunologically competent cells in delayed type hypersensitivity reactions.

The specifically active cells may form a small proportion of the injected cells, which might make it statistically impossible to detect any accumulation of active cells above the normal background of unsensitized donor cells. In addition, one should consider the possibility that the cells which transfer the delayed type hypersensitivity reaction are lysed after contact with antigen. Considering these points, the increased percentage of labelled cells after 6 hours, although not statistically significant, could be meaningful.

It was shown that the great majority of lymphoid cells in the lesion were labelled, when delayed type hypersensitivity reactivity was passively transferred with unlabelled cells in guinea pigs previously treated with tritiated thymidine (2). Consequently most of the infiltrative cells in the lesion of passively transferred delayed type hypersensitivity reactions must be of recipient origin.

SUMMARY

Tritiated thymidine labelled tubercle bacilli sensitized lymphoid cells were injected intravenously into guinea pigs and a delayed type hypersensitivity reaction was produced in the lung by the inhalation of PPD aerosol. Lungs were excised 3, 6, 24 and 48 hours after PPD inhalation and the number of cells labelled with tritiated thymidine was counted after radioautography. The number of labelled cells was found to be slightly increased 6 hours after PPD inhalation, though this was not statistically significant. Only 19 to 27 per cent of the transferred cells were labelled and these were not necessarily the immunologically competent cells. The increased percentage of labelled cells after 6 hours could be meaningful.

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