Delayed Hypersensitivity in Man: Transfer by Lymphocyte Preparations of Peripheral Blood

Abstract. *Eight passive transfers of delayed skin hypersensitivity to pollen antigens were achieved by injection of lymphocyte preparations of peripheral blood from sensitized donors into previously nonreactive subjects. The granulocytes and platelets were removed from the blood of each donor by passage through a column of glass beads coated with silicone.*

It is widely inferred that cells of the lymphoid series found in the peripheral blood of man are capable of transferring delayed hypersensitivity (*1, 2*), although there is no direct evidence for this belief. This inference appears to be based on three lines of evidence: (i) the demonstration in animals that mononuclear cells from peritoneal exudates, lymph nodes, and spleen are capable of transferring delayed hypersensitivity (*3*); (ii) the failure of living granulocytes to transfer tuberculin sensitivity in the guinea pig (*4*); and (iii) the demonstration in man that mixed peripheral leucocyte suspensions from sensitive subjects are able to transfer delayed hypersensitivity to previously insensitive subjects. In addition, Braunsteiner, in 1958 (*5*), showed that transfer of sensitivity to tularin and tuberculin could be achieved in man by tissue lymphocytes gathered by a skin window technique. Since the first description of the successful transfer of tuberculin sensitivity in man by circulating leucocytes (*6*), a wide variety of sensitivities has been transferred by this technique, including that to streptococci (*7*), fungi (*8*), viruses (*9*), chemicals (*2*), and pollens (*10*). In addition, cell-free extracts prepared from mixed peripheral leucocytes of sensitized subjects have been used successfully in transferring delayed hypersensitivity in man (*11*). By employing a column technique for the separation of lymphocytes from the other white cell species, we have shown directly that cells indistinguishable from the human peripheral lymphocyte are both sufficient and specific for the passive transfer of induced delayed hypersensitivity to timothy and ragweed pollens.

Samples of venous blood, 200 to 250 ml, were drawn from male prisoners who had a strongly positive intradermal skin reaction at 24 hours to a 1 : 1000 dilution of timothy or ragweed antigen. This sensitivity had been previously induced by injecting the timothy or ragweed antigen in a water-in-oil emulsion into nonatopic subjects (*10, 12*). All donors had been subjected to a skin test within 2 weeks prior to venesection. The blood was mixed with 2 ml of heparin (1000 units/ml) and passed through a large column of glass beads coated with silicone at 36°C (*13*). The usable effluents ranged from 150 to 175 ml, and consisted of whole blood from which most of the platelets and granulocytes had been removed (*14*). The effluent was then mixed with a half volume of 1.5 percent ethylenediaminetetraacetic acid disodium salt (EDTA) in 0.7 percent NaCl. The separation of lymphocytes from erythrocytes was then carried out by a sedimentation method in which polyvinylpyrrolidone was used (*15*). The lymphocyte concentrate was transferred to a 50-ml screw-top centrifuge tube and centrifuged at 800*g* for 10 minutes. The supernatant was discarded and the cellular button washed twice with a solution consisting of 25 ml of 0.9 percent NaCl, 1 ml of 1.15 percent KCl, 2 ml of 0.1*M* phosphate buffer of *p*H 7.4, and 0.25 ml of 0.11*M* EDTA. The cells were washed a third time in the same buffered salt solution without EDTA and finally suspended for injection by agitation in a small volume of this solution. Special care was taken in making slide preparations of these suspensions, as ordinary smears made at room temperature lacked clear cytological detail. It was found that daubing the chilled suspensions on chilled slides in the 5°C cold room followed by immediate drying at room temperature with a current of air resulted in good retention of morphology. Equal volumes of this cell suspension were then injected intradermally and subcutaneously into the deltoid areas of a male recipient who had been demonstrated to be negative to ragweed and timothy pollen antigens within the previous 2 weeks. Intradermal challenge with a 1 : 1000 dilution of the aqueous antigen or antigens was performed 6 days later at a distal site on the forearm. Sites of challenge were observed at 20 minutes and at 24 hours. Reactions were considered positive if regions of definite erythema and induration of at least 5 mm in diameter were found at 24 hours.

Figure 1 is a black-and-white reproduction of an original color transparency made of a typical passively transferred, delayed skin reaction 24 hours after intradermal injection of 0.05 ml of 1 : 1000 extract of timothy pollen. The area on the forearm which appears dark in Fig. 1 appears on the original as a striking reddish discoloration. All subjects were observed for 20 minutes after challenge and in no case was an immediate skin reaction observed. Skin biopsies were performed on four of the eight positive delayed skin reactions described below, and microscopic examination of the stained sections showed marked perivascular infiltration by mononuclear cells, consistent with the cellular reaction typically found in delayed hypersensitivity (*16*). The duration of the transferred sensitivity was followed in some cases. One recipient has maintained a positive skin test for 9 months.

The results are summarized in Table 1. Nonspecific activation resulting from the transfer procedure per se as a possible cause of successful trans-

Table 1. Results of delayed hypersensitivity transfer experiments. The extent of the erythema and induration of the skin reactions were graded as follows: 4+, 20 mm; 3+, 15 to 20 mm; 2+, 10 to 15 mm; 1+, 5 to 10 mm; 0, to 0 to 5 mm. The recipients had been demonstrated to give negative reactions to both ragweed and timothy pollen antigens within the 2 weeks prior to transfer.

Expt.	Skin sensitivity of donor		$10^7 \times$ No. of lymphocytes in blood drawn	Lymphocytes in injected suspension		New sensitivity of recipient after transfer	
	Ragweed	Timothy		$10^6 \times$ No.	%	Ragweed	Timothy
1	0	4+	48	150	95		2+
2	0	2+	101	132	98		0
3	0	2+	63	126	99		0
4	0	4+	53	75	98		2+
5	0	4+	53	78	98		1+
6	0	4+	74	66	97		0
7	0	4+	76	142	98		2+
8	0	4+	37	97	98	0	2+
9	4+	0	46	110	97	2+	0
10	4+	0	60	80	94	2+	0
11	2+	0	63	65	98	0	0
12	4+	0	67	102	98	2+	0

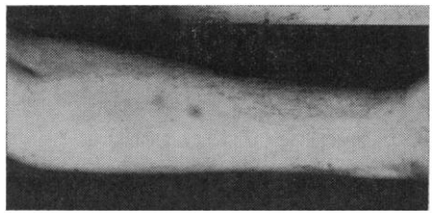

Fig. 1. Black-and-white reproduction of an original color transparency made of a typical passively transferred, delayed skin reaction to extract of timothy pollen as it appeared 24 hours after intradermal challenge. The darkening adjacent to the reaction is due to a previous test with an unrelated antigen.

fer would seem to be ruled out by the results of the specific transfer experiments (Expts. 8, 9, 10, and 12) and by the several failures encountered (Expts. 2, 3, 6, and 11). Evidently, successful transfer depends both on the number of cells injected and the degree of sensitivity of the donor. Thus the only failure with a 4+ donor occurred when 66 million cells were injected (Expt. 6), and the other three failures all occurred with donors who were only 2+ (Expts. 2, 3, and 11). Taken together, these results show that typical delayed hypersensitivity has been transferred by the injected cells and that the reaction is specific for the antigen to which the donor is sensitive.

Table 1 also shows the numbers and purity of the cell populations injected into the recipients. Microscopic examination of the daub preparations revealed that the cell populations consisted of from 94 percent to 99 percent of cells morphologically indistinguishable from the peripheral lymphocyte. The vast majority of these appeared to be small lymphocytes. A few polymorphonuclear neutrophils and rare basophils and eosinophils made up the balance of the cells seen, while occasional monocytes could not be definitely excluded. Very few platelets were seen on slide preparations from any of the injected cell suspensions. Quantitative data obtained on the lymphocytes in the blood of the column effluents showed that from 37 to 90 percent of the lymphocytes in the donor's blood were recovered in the effluents, whereas in the final suspensions used for injection only from 9 to 31 percent of the lymphocytes were recovered. It is evident that segregation of subpopulations of lymphocytes during the two fractionation steps cannot be excluded. The details of the washing procedure are of interest because the final suspensions showed no aggregates when they were observed on the white cell counting chamber, and the distribution of cells permitted an accurate and reproducible count as well as facilitated the morphological examinations. The fact that the lymphocytes were washed three times decreases the probability that a soluble factor from disrupted platelets, polymorphonuclear neutrophils, or other minor cellular components could remain adherent to the lymphocytes and thus confer upon them the ability to transfer.

In conclusion, while our experiments do not prove that the small lymphocytes of the peripheral blood are necessary for the transfer of delayed hypersensitivity to timothy grass and ragweed antigens in man, they do show that some preparations of circulating lymphocytes from sensitized subjects are both sufficient and specific for the transfer.

RAYMOND G. SLAVIN
JAMES E. GARVIN
Departments of Internal Medicine and Biochemistry, Northwestern University Medical School, Chicago 11, Illinois

References and Notes

1. M. W. Chase, *J. Allergy* **26**, 247 (1955); H. S. Lawrence, in *Cellular Aspects of Immunity: Ciba Found. Symp.* G. E. W. Wolstenholme and M. O'Connor, Eds. (Little, Brown, Boston, 1960), p. 277.
2. W. L. Epstein and A. M. Kligman, *J. Invest. Dermatol.* **28**, 291 (1957).
3. K. Landsteiner and M. W. Chase, *Proc. Soc. Exptl. Biol. Med.* **49**, 688 (1942); M. W. Chase, in *Cellular and Humoral Aspects of Hypersensitive States*, H. S. Lawrence and Paul B. Hoeber, Eds. (Harper, New York, 1959), p. 251; M. N. Metaxas and M. Metaxas-Buehler, *J. Immunol.* **75**, 333 (1955).
4. W. F. Kirchheimer, A. R. Hess, R. G. Spears, *Am. Rev. Tuberc. Pulmonary Diseases* **64**, 516 (1951).
5. H. Braunsteiner, J. Paertan, N. Thumb, *Blood* **13**, 417 (1958).
6. H. S. Lawrence, *Proc. Soc. Exptl. Biol. Med.* **71**, 516 (1949).
7. ———, *J. Immunol.* **68**, 159 (1952).
8. F. T. Rapapport, H. S. Lawrence, J. W. Millar, D. Pappagianis, C. E. Smith, *ibid.* **84**, 358 (1960).
9. W. J. Warwick, A. Page, R. A. Good, *Proc. Soc. Exptl. Biol. Med.* **93**, 253 (1956).
10. R. J. Becker, D. B. Sparks, S. M. Feinberg, R. Patterson, J. J. Pruzansky, A. R. Feinberg, *J. Allergy* **32**, 229 (1961).
11. H. S. Lawrence, *J. Clin. Invest.* **34**, 219 (1955).
12. S. M. Feinberg, R. J. Becker, R. G. Slavin, A. R. Feinberg, D. B. Sparks, *J. Allergy* **33**, 285 (1962).
13. J. E. Garvin, in preparation.
14. ———, *J. Exptl. Med.* **114**, 51 (1962).
15. P. Gold and M. Cole, *J. Lab. Clin. Med.* **56**, 310 (1960).
16. P. G. H. Gell, *Cellular and Humoral Aspects of the Hypersensitive States*, H. S. Lawrence and Paul B. Hoeber, Eds. (Harper, New York, 1959), p. 49.
17. Supported in part by research grant AI-04232 from the National Institute of Allergy and Infectious Diseases, and in part by USPHS career development award (No. 238) and National Cancer Institute grant No. 06681, to James E. Garvin. The authors thank E. Stephen Kurtides, and Walter A. Rambach of Northwestern University Medical School for their review of the final slides. We also wish to acknowledge the splendid cooperation in this study of Warden Frank J. Pate, the administrative staff, and inmates of the Illinois State Penitentiary, Stateville.

15 May 1964

Benzo(a)pyrene and Other Polynuclear Hydrocarbons in Charcoal-Broiled Meat

Abstract. *The possible production of carcinogenic polynuclear hydrocarbons in the charcoal broiling of food has been investigated. Fifteen steaks were cooked and the polynuclear compounds were extracted, separated by chromatography, and identified spectrometrically. Many polynuclear hydrocarbons were identified, but no nitrogen heterocyclic compounds were detected. The carcinogen benzo(a)pyrene was present in the average amount of 8 micrograms per kilogram of steak.*

It has been suggested many times that high-temperature cooking of food might give rise to carcinogenic hydrocarbons of the polynuclear aromatic group. Indeed, there has been one report of the finding of dibenz(*a,h*)anthracene in charcoal-broiled meat (*1*). Five years ago an analysis of charcoal-broiled steak was carried out in this laboratory and several polynuclear hydrocarbons were separated and identified, including pyrene, fluoranthene, chrysene, benz(*a*)anthracene, and benzo(*g,h,i*)perylene. The analytical method used was cumbersome and lengthy, involving alkaline and acid hydrolyses occupying several weeks, and undoubtedly introduced serious losses, particularly of any unstable hydrocarbons present. The experiment has been repeated with a simpler and more sensitive method now available (*2*).

Fifteen steaks were charcoal broiled, the outer layers were removed and extracted, the polynuclear material was extracted from the lipids, and the polynuclear aromatic hydrocarbons were separated by column and paper chromatography. Identification and measurement were made by ultraviolet absorp-