Delayed Hypersensitivity: Bone Marrow as the Source of Cells in Delayed Skin Reactions

Abstract. *Adult Lewis rats were thymectomized, irradiated, and restored with bone marrow from allogeneic (or F_1) donors. They were passively sensitized to tuberculin by a subsequent transfer of Lewis lymph node cells and were given intradermal skin tests with tuberculoprotein. In 24-hour skin reactions the majority of cells, in successive experiments, were shown to be allogeneic (or F_1) with the use of isoantibody against the antigens of the transplanted marrow cells and by the indirect fluorescent antibody technique. Our results demonstrate that the nonspecific cells making up a large proportion of the infiltrating elements in tuberculin skin reactions probably originate in the bone marrow.*

The cells that infiltrate perivascular regions in reactions of delayed (cellular) hypersensitivity are largely hematogenous and are derived from a rapidly dividing population; this has been shown with the use of H^3-thymidine labeling in various delayed skin reactions (*1*), in autoallergic encephalomyelitis (*2*) and thyroiditis (*3*), and in rejection of skin homografts (*4*). Similar labeling experiments, combined with passive transfer, have established the fact that specifically sensitized cells, probably coming from lymph nodes which drain the site of immunization, make up a small minority of infiltrating cells in the lesions (*5*); these in turn may come from uncommitted small lymphocytes derived from the thymus (*6*). The majority of cells in the infiltrate are phagocytic and resemble morphologically the "histiocytes" or "macrophages" appearing at sites of nonspecific inflammation (*7*). The latter are derived more or less directly from the bone marrow by way of the blood stream (*8*). We have tried to determine whether the nonspecific cells found at the sites of typical delayed reactions were also derived from the bone marrow.

Inbred Lewis rats (L) (*9*) were thymectomized at 5 to 7 weeks of age and were x-irradiated (900 rads, total body) at 8 to 10 weeks. Immediately after the irradiation, they were intravenously infused with 2×10^8 to 4×10^8 normal bone marrow cells from donors of BN (*9*), DA (*10*), (BN × L) F_1, or (DA × L) F_1 strains. Seven days later the recipients were given 4×10^8 to 6×10^8 sensitized lymph node cells from Lewis donors immunized 9 days earlier with heat-killed tubercle bacilli suspended in oil. Twenty-four hours after transfer of the lymphocytes, the animals received skin tests with 50 μg of tuberculin purified protein derivative (specific) (*11*) and with 20 percent turpentine in olive oil (nonspecific). The reactions were observed at 24 hours, and the sites of the skin tests as well as samples of the bone marrow, spleen, and mesenteric and axillary lymph nodes were removed for study. Cells derived from the donor marrow were detected by a modified indirect fluorescent antibody technique (*12*). Suspensions of live cells were incubated with Lewis isoantiserums against BN or DA antigens, washed, and treated with fluorescein-conjugated rabbit antiserum against rat γ-globulin (*9*). The conjugate was neither diluted nor absorbed. The use of suspensions of live, unfixed cells made absorption of the fluorescent serums unnecessary, as there was no nonspecific staining (*12*). The cells were washed again and examined for fluorescence. For most tissues, over 100 cells were counted, and the percentage of fluorescent cells was calculated.

In recipients of allogeneic (BN or DA) bone marrow there was considerable variability in the size of the skin reactions and in the yield of cells from the reaction sites (Table 1). In three of the four animals tested, the majority of cells in the reaction were derived from the infused bone marrow. The bone marrow itself was almost entirely of donor type, while the spleen and lymph nodes contained a much smaller proportion of cells derived from marrow. The single recipient of DA cells showed signs suggestive of a graft-versus-host reaction; in this animal, donor cells were present in high proportion in all tissues studied. More consistent results were obtained in rats given bone marrow from F_1 hybrids (Table 2). Cells from hybrid donors presumably do not encounter foreign antigens in the recipient and participate normally in physiological and immunological functions. In these recipients, two-thirds or more of the infiltrating cells in tuberculin reactions were found to be derived from marrow. Nonspecific inflammatory reactions induced by turpentine consistently had high percentages of marrow-derived cells, as Volkman and Gowans observed (*8*). The percentages of such cells appeared parallel in the two types of reactions.

Our results confirm the earlier finding (*5*) that the majority of cells accumulating at the site of a delayed (tuberculin) reaction, under the special circumstances of the passive transfer experi-

Table 1. Origin of infiltrating cells in specific and nonspecific skin reactions elicited in rats given F_1 hybrid bone marrow. Numbers in parentheses are means.

Strain donor marrow	Tuberculin reaction*	Fluorescent cells in recipient (%)					
		Reaction sites		Lymphoid tissues			
		Purified protein derivative	Turpentine	Bone marrow	Spleen	Mesenteric lymph node	Axillary lymph node
BN	8+	55.5		92.5	19.8	22.7	10.8
BN	12+	25.0		85.9	23.7	27.0	21.8
DA†	8±	88.7		95.6	60.5	82.2	64.5
BN	14+	59.0	77.4	90.7	34.5	36.8	16.7
	(10.5)	(57.1)	(77.4)	(91.2)	(34.6)	(42.2)	(28.4)

* Diameter (mm) and degree of induration, measured subjectively on scale of 0 to +++. † Probable graft-versus-host reaction.

Table 2. Origin of infiltrating cells in specific and nonspecific skin reactions elicited in rats given F_1 hybrid bone marrow. The numbers in parentheses are means.

Strain donor marrow	Tuberculin reaction*	Fluorescent cells in recipient (%)					
		Reaction sites		Lymphoid tissues			
		Purified protein derivative	Turpentine	Bone marrow	Spleen	Mesenteric lymph node	Axillary lymph node
(DA × L)	15++	79.0		98.1	38.1	16.0	14.0
(DA × L)	17++	66.0	86.7	95.0	33.6	14.3	10.7
(BN × L)	11±	86.0	86.5	97.5	25.7	27.0	
(DA × L)	17+±	75.5	84.2	94.3	29.3	25.5	28.2
	(15.0)	(76.6)	(85.8)	(96.2)	(31.6)	(21.2)	(17.6)

* Diameter (mm) and degree of induration determined as for Table 1.

ment, are derived from the host. The fluorescent-antibody technique we used has an advantage over labeling with H^3-thymidine or chromosome marker techniques in that all cells of a given population, rather than just dividing cells, may be detected.

Our study is the first demonstration that these cells come from the bone marrow. Volkman and Gowans (*8*) have shown that phagocytic cells appearing at sites of nonspecific inflammation come directly from a dividing cell population in the bone marrow, and this finding was confirmed in our study by results obtained with turpentine. It is a reasonable inference that the same cell population participates in both specific and nonspecific reactions, and this hypothesis is supported by the fact that the percentages of marrow-derived cells found in each are similar. Thus the delayed reaction is comparable to the ameboid cell reaction in starfish larvae pricked with a rose thorn (*13*), differing only in the specificity of the trigger and the greatly increased intensity of the response.

The possibility that cells originating in the bone marrow sojourn in another organ before traveling to specific skin reaction sites appears to be ruled out by the discrepancy between their percentage in these sites and their percentage in peripheral lymphoid organs, the lymph nodes in particular. Our experiments exclude the thymus as a participant in the production or processing of these cells. Thus they are completely distinct from the population of specifically sensitized cells, which appear to originate in the marrow, pass through the thymus into the peripheral pool of immunocompetent cells, and undergo immunization in lymph nodes (*6*, *14*).

Our experiments, while they show that cells from the bone marrow can act as the source of cells participating in delayed skin reactions, do not establish unequivocally that they do so under normal physiological conditions. Conclusive proof of this relationship must be obtained in future investigations.

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Membranes in Polyribosome Formation by Rabbit Reticulocytes

Abstract. *When rabbit reticulocytes are incubated with* n-*butanol, an agent disruptive to the structure and function of cellular membranes, there is a rapid disaggregation of the polyribosomes. Reaggregation is promoted when the* n-*butanol is diluted below a critical concentration or when the cells are washed free of the alcohol and the incubation is continued. Neither disaggregation nor regeneration will occur in the absence of protein synthesis. These observations suggest that integrity of the reticulocyte membrane is necessary for the attachment of ribosomes to messenger RNA and for the formation of polyribosomes.*

Polyribosomes in rabbit reticulocytes disaggregate when the cells are incubated in the presence of tryptamine (*1*), homotryptamine, and α-ethyltryptamine (*2*). Baglioni and Colombo (*1*) indicated that tryptamine may act as other than an antagonist of tryptophan, and Hori *et al.* (*2*) suggested that the activity of these compounds may be related to some property of indoleamines. We have found that another heterocyclic amine, primaquine, causes disaggregation of polyribosomes in concentrations lower than those causing hemolysis. Primaquine induces changes in the permeability of erythrocyte membranes, resulting first in a loss of intracellular potassium and, in higher concentrations, in complete hemolysis (*3*). Therefore, it seems reasonable to assume that these various organic amines might cause polyribosome disaggregation by altering a cell membrane structure or functional component.

n-Butanol is another compound that induces changes in the permeability of erythrocyte membranes (*4*). At a concentration of 0.4 mole/liter, it causes a reversible loss of intracellular potassium and a gain in sodium ions (*5*) without inhibiting glycolysis (*6*). Since *n*-butanol is a dipolar and lipid-soluble molecule, it becomes concentrated in the lipid part of lipoprotein membranes of cells and brings about a disorientation of membrane structure and loss of function (*7*). *n*-Butanol appeared to be the agent of choice in an investigation of the role of membrane integrity on polyribosome formation, since its effects on cell function are considered to be a direct result of its action on membranes.

When rabbit reticulocytes were incubated in 0.1*M* *n*-butanol at 37°C, the polyribosomes disaggregated to monomeric ribosomes within 5 minutes (Fig. 1). No effect was observed if the concentration of the *n*-butanol was below 0.05 mole/liter. We prevented polyribosome disaggregation by inhibiting protein synthesis with cycloheximide (*8*) or by retarding the rate of protein synthesis by omitting the essential amino acid histidine (*2*, *9*). In the absence of *n*-butanol this concentration of cycloheximide or the omission of histidine had no effect on the ribosome-polyribosome profile. If the cells were treated with 0.1*M* *n*-butanol at 0°C no polyribosome disaggregation occurred; indeed, at this temperature a concentration of 0.4 mole/liter was ineffective. Polyribosomes were reformed either when the butanol was diluted with additional buffered medium or when the cells were washed with the medium and the incubation was continued at 37°C for 10 minutes (Fig. 1). This regeneration did not occur if protein synthesis was inhibited by cycloheximide, or if the cells were maintained at 0°C.