Graft Versus Host Activity of Bone Marrow Lymphocytes

Large numbers of small lymphocytes are present in the bone marrow parenchyma of laboratory mammals but their functional potential remains uncertain[1]. Some lymphocytes separated from the bone marrow of Lewis strain rats have recently been shown to transform into proliferating blast-like cells when cultured in vitro with F_1 (Lewis and Brown, Norway) hybrid rat lymphocytes[2]. In such a mixed lymphocyte culture the blastogenic response is confined to the parental strain small lymphocytes and is generally regarded as an immune reaction to the foreign antigens of the F_1 hybrid cells[3]. However, there has been no direct evidence that marrow lymphocytes can participate in immune reactions against allogeneic cells in vivo. Therefore, in the present studies the responsiveness of rat marrow lymphocytes to allogeneic cells in vivo was tested by their ability to induce a graft versus host (GVH) reaction. The experiments used a lymph node weight method to assess GVH activity[4,5] together with a fractionation technique previously developed to separate high concentrations of lymphocytes from marrow cell suspensions[6,7].

The GVH assay system was standardized using cell suspensions of recognized GVH activity. Doses of 40×10^6 spleen cells from 3–5-month-old male Lewis rats were injected into the hind footpads of 2–3-month-old male F_1 (Lewis and Brown, Norway) hybrid rats according to the technique of LEVINE[4]. In groups of F_1 hybrids taken at close intervals thereafter the weight of the popliteal lymph nodes draining the injection sites, initially 6.3 ± 0.3 mg, increased exponentially for 6 days, plateaued (190–210 mg) at 8–12 days and then declined. Thus, there was an approximately 30-fold increase in popliteal node weight by the 8th day. Large dividing basophilic cells appeared in these lymph nodes in increasing numbers from 2 days onwards. Pelvic and lumbar nodes showed weight increases that were smaller and more variable than those of the popliteal nodes, while contralateral popliteal nodes draining the uninjected feet remained unchanged. From these experiments the popliteal node weight 8 days after footpad inoculation was chosen as the optimum assay system.

Popliteal nodes showed either no change or only minimal weight increase 8 days after the inoculation of 1. heat-inactivated spleen cells, liver cells or erythrocytes from Lewis rats into F_1 hybrid recipients, or 2. 20×10^6 to 200×10^6 spleen cells from Lewis rats into syngeneic Lewis recipients (Table). Lymph node enlargement occurred only when viable lymphoid cells of the parental strain were injected into F_1 hybrid hosts, i.e. the enlargement was dependent upon GVH activity of the parental strain lymphocytes.

The weight of the popliteal lymph node was found to be a sensitive and highly reproducible parameter of localized GVH activity in the rat. The table compares the efficacy of several Lewis rat cell suspensions in inducing popliteal node enlargement when injected into the hind footpads of F_1 (Lewis and Brown, Norway) hybrid rats. Doses of 60×10^6 to 200×10^6 nucleated bone marrow cells, of which approximately 20% were small lymphocytes, produced significant popliteal node enlargement. Lymphocyte-rich fractions separated from Lewis rat marrow suspensions by centrifugation in sucrose-serum density gradients[2,6,7] showed a greater effect than whole marrow on lymph node weight in doses of 20×10^6 to 60×10^6 cells. This enhancement, suggesting an enrichment of cells capable of GVH activity in lymphocyte-rich marrow fractions, can be correlated with the content of small lymphocytes. The slowly-sedimenting lymphocyte-rich marrow fractions used in the present experiments consisted mainly of lymphoid cells ($83.6 \pm 2.9\%$) of which more than 80% were small lymphocytes. The main contaminants were relatively mature granulocytes and late erythroblasts. Lymphocyte-rich marrow fractions were considerably more effective than thymus cell suspensions in producing popliteal node enlargement (Table).

The lymph node enlargement obtained with lymphocyte-rich marrow fractions could not be attributed to circulating lymphocytes in the marrow blood. It has been estimated in guinea-pigs that appreciably less than one in 2000 small lymphocytes in marrow cell suspensions are derived from marrow blood[2]. Assuming a similar value in the rat, the doses of 20×10^6 lymphocyte-rich marrow fraction cells used in the present experiments contained fewer than 0.007×10^6 blood small lymphocytes, yet the resultant popliteal node enlargement was similar to that produced by 1×10^6 blood leucocytes (containing approximately 0.7×10^6 small lymphocytes). Doses of 0.1×10^6 blood leucocytes consistently failed to produce popliteal node enlargement. Since, in addition, the donor rats were routinely exsanguinated before the marrow was sampled, the contaminating blood lymphocytes were clearly too few to account for the effect of lymphocyte-rich marrow fractions on popliteal node weight.

Wet weight of popliteal lymph nodes of Lewis and F_1 (Lewis and Brown, Norway) hybrid rats 8 days after the footpad injection of cells from Lewis rats

Recipient animals[a]	Donor Lewis cells[b] Source	Number	Recipient popliteal lymph node weight (mg)[c]
F_1 (Lewis and Brown, Norway) hybrid rats		0	6.3 ± 0.3
	Spleen	5×10^6	35.6 ± 3.8
		40×10^6	188.0 ± 11.9
	Bone marrow	60×10^6	13.7 ± 0.6
		200×10^6	37.8 ± 5.0
	Lymphocyte-rich marrow fraction	20×10^6	18.0 ± 2.6
		60×10^6	51.3 ± 7.9
	Thymus	60×10^6	11.6 ± 1.9
		200×10^6	31.8 ± 2.8
	Blood leucocytes	1×10^6	18.5 ± 1.5
		10×10^6	108.2 ± 19.2
Lewis rats	Spleen	20×10^6	5.5 ± 0.2
		200×10^6	8.8 ± 0.7

[a] Based on 27 popliteal lymph nodes from normal F_1 (Lewis and Brown, Norway) hybrid rats and groups of 3–11 determinations in either F_1 (Lewis and Brown, Norway) hybrid or Lewis rats given footpad injections of Lewis cells. [b] Cell suspensions in Hank's balanced salt solution were prepared from the spleen, bone marrow and thymus as described elsewhere[2]. Blood leukocyte suspensions were obtained from heparinized cardiac blood following erythrocyte sedimentation with 6% fibrinogen. [c] Mean ± standard error.

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The results provide direct evidence that certain parenchymal marrow lymphocytes can produce GVH lymph node enlargement. In current experiments highly labelled large blast-like cells have been seen in radioautographs of F_1 hybrid popliteal lymph nodes 2–3 days after the footpad injection of H^3-uridine labelled cells from lymphocyte-rich marrow fractions of parental strain rats. Some of the marrow lymphocytes therefore appear to be capable of blastogenic transformation in response to histocompatibility antigens in vivo as well as in vitro.

The great majority of small lymphocytes in the bone marrow of rats and guinea-pigs are newly-formed, locally-produced cells, which have a short intramyeloid life span and migrate continuously via the blood stream to the spleen and lymph nodes[1,8–10]. The marrow is therefore a major site of small lymphocyte production. If the marrow cells which produce GVH reactions are among this population of newly-formed small lymphocytes the marrow may provide a continous source of small lymphocytes capable of reacting against foreign cells in the blood and other peripheral lymphoid tissues. On the other hand, recent radioautographic studies in this laboratory as well as those of other investigators[11] have indicated the presence of a small number of long-lived small lymphocytes in rat marrow. Further radioautographic studies are therefore required to determine whether the cells possessing GVH activity might be contained within this latter subpopulation of small lymphocytes in the marrow[12].

Résumé. Des suspensions de cellules provenant de la moëlle osseuse, ou des fractions cellulaires d'une moëlle osseuse d'une souche parentale ont été injectées dans la plante des pieds de rats hybrides (F_1). Le poids des nodules lymphatiques poplités constitue un test sensible de la réaction greffe-receveur qui résulte de l'injection des cellules et montre qu'une telle réaction peut être provoquée par certains lymphocytes provenant du parenchyme de la moëlle.

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Studies on Glucose-6-Phosphate Dehydrogenase: Variability in ATP Inhibition[1]

Glucose-6-phosphate dehydrogenase (G-6-PD) activity is tremendously important to erythrocyte integrity. Where activity is decreased, the erythrocyte is unusually susceptible to 'oxidant' drugs, which may markedly shorten its in vivo life[2]. The enzyme is inhibited by adenosine-triphosphate (ATP)[3], various steroids[4,5], and palmityl-CoA[6,7].

The effect of ATP on G-6-PD activity is particularly important because the inhibition constant (K_i) is within fluctuating levels of intracellular ATP concentration and because ATP helps regulate glycolysis by inhibiting fructose-6-phosphate kinase[8].

A discrepancy between G-6-PD activity of sheep hemolysates (measured under optimum spectrophotometric conditions) and the ability of erythrocytes to reduce glutathione[9], suggested studying effects of ATP on sheep erythrocyte G-6-PD.

Materials and methods. All blood samples, except one human sample (an aliquot of citrated blood 4 weeks in storage), were collected using heparin as an anticoagulant.

Glucose-6-phosphate-dehydrogenase activity was measured by following the reduction of nicotinamide-adenine-dinucleotide phosphate (NADP) at 340 nm recorded on a Gilford automatic recording spectrophotometer. The reaction mixture for hemolysates (1.0 ml) contained 0.2 m*M* NADP, 0.1 *M* *Tris*-buffer (pH 8.0), $MgCl_2$ and 0.6 m*M* glucose-6-phosphate at 25 °C[10].

Glucose-6-phosphate dehydrogenase was partially purified, using previously described techniques[10]; all preparations were dialyzed overnight against *Tris* 105 m*M*, pH 8.0; 2.7 m*M* EDTA; 7 m*M* β-mercapto-ethanol and 10 μ*M* NADP prior to the experimentation. For kinetic studies the reaction mixture was lowered to pH 7.35, the temperature raised to 30 °C, $MgCl_2$ omitted, and variable amounts of ATP (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 m*M*) and glucose-6-phosphate (0.0125, 0.025, 0.05, 0.01, 0.3, 0.4, and 0.6 m*M*) were used[3]. The reaction was initiated by adding the enzyme as the last component. The Michaelis-Menten constant (*Km*) for glucose-6-phosphate, the inhibition constant, and their respective standard errors were calculated using a least square fit to the Michaelis-Menten equation[11], assuming the inhibition competitive and observed velocities equal in variances.

Results. Competitive inhibition was not observed using the sheep enzyme (Table). The reaction rates with ATP were in the expected range for samples without ATP.

Observed *Km* values for both human and sheep G-6-PD are higher than previously reported[9,10,12] – probably because we lowered the pH of the assay mixture and used a higher temperature. The *Ki* value for 1 sample (human, 1) was approximately half that observed in the other 2 samples; hemolysate activity of that one was approximately 20% of normal (1.6 μm NADP/min/g

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