Bone Marrow: The Bursa Equivalent in Man?

Abstract. *Human bone marrow lymphoid cells, particularly when enriched with plasma cells, as in multiple myeloma, respond to pokeweed mitogen and to antiserum to immunoglobulin but not to phytohemagglutinin. Cells of patients with X-linked agammaglobulinemia of the bursal deficient type failed to respond to either pokeweed or to the antiserum to immunoglobulin. Leukocytes of the agammaglobulinemia patients however responded in a normal fashion to phytohemagglutinin. Just as the in vitro response to phytohemagglutinin is taken as an index of the thymus-dependent system, the in vitro response to both antiserum to immunoglobulin and pokeweed may be considered an index for the bursal-dependent system. Human bone marrow, therefore, contains bursal cells and probably very few or no thymus cells.*

Immunocompetent cells are either thymus dependent (TD) or bursal dependent (BD) (*1*). The former is responsible for cell-mediated immunity and the latter for humoral immunity (*1*). Though it has been uniformly accepted that BD cells are in or derived from the bone marrow in the mouse (*2*), the equivalent organ in man and other species is still unknown (*2*). There is some evidence that the bursa equivalent in man may be related to the tonsil or the gut-associated lymphoid cells (or both) (*3*). The lack of correlation between the various clinical syndromes with defective humoral responses (BD cell deficiency) and the status of the gut-associated lymphoid cells (*3*) leaves doubt as to the validity of this assumption and indicates that further search for that organ in man is necessary. Perey *et al.* and Henry *et al.* have shown that in the rabbit the gut-associated lymphoid cells play an essential role in the humoral responses to certain antigens (*4*); however, we have shown that these organs probably contain memory cells and not cells responsible for production of antibody in true primary immune responses (*5*).

Some investigators have indicated that BD cells carry immunoglobulin (Ig) receptors on their surface, but that TD cells lack these receptors (*6*). We and others have shown that patients with deficient BD humoral responses lack these Ig receptors on their leukocytes (*6, 7*).

The responses in vitro of BD or TD cells to the nonspecific mitogen phytohemagglutinin (PHA) or to pokeweed mitogen (PWM) show differential results. PHA stimulates TD cells but not bone marrow cells (*8*). However, PWM stimulates BD cells and plasmacytoma cells and is capable of inducing plasmacytosis after administration in vivo (*9*).

In our study we have used the abovementioned functional criteria: the presence of Ig receptors on cells and the in vitro response to PWM to show that human bone marrow cells satisfy these two criteria and may be considered the bursa equivalent in man. We have studied bone marrow cells (BM) and peripheral blood leukocytes (WBC) of 12 patients with multiple myeloma, 2 patients with X-linked agammaglobulinemia (Bruton type), and 16 controls. Seven of the controls were patients with iron-deficiency anemia but with normal Ig levels (Table 1). Multiple BM aspirates or peripheral WBC were collected (*10*). In order to separate the plasma cells or lymphocytes, the collected cells were applied on sucrose gradients (*11*). The in vitro culture system is similar to that described by Daguillard *et al.*, with a few modifications (*12*). The method of McConahey and Dixon (*13*) was used for labeling the antiserum to immunoglobulin with ^{125}I. The antiserum to Ig was purchased (Hyland Laboratories) and processed (*14*). The various mitogens PHA-M (Difco) and PWM (Grand Island) were diluted in medium 199 (Microbiological Associates) before use.

Table 2 indicates the response in vitro of bone marrow or leukocytes to PHA and PWM. Whereas leukocytes did respond to both PHA and PWM in myeloma cases and the controls, the bone marrow cells failed to respond to

Table 1. Patient material used for the study.

Diagnosis	Patients (No.)	Cells studied		Immunoglobulins* (mg/100 ml)			Plasma cells in BM aspirate (%)
		BM	WBC	IgG	IgM	IgA	
IgG myeloma	9	+	+	1900–4600	0–140	85–220	19–47
IgA myeloma	3	+	+	500–1400	30–85	1400–4900	28–41
X-linked agammaglobulinemia	2	+	+	0	0	0	0
Iron-deficiency anemia	7	+	+	1100–1520	70–110	195–210	0–5
Normal	9	ND†	+	1350–1700	95–105	200–205	ND

* Serum immunoglobulins were determined by the quantitative immunoplates (Hyland). † Not done.

Table 2. The in vitro proliferative effects of phytohemagglutinin (PHA-M) or pokeweed mitogen (PWM) on peripheral leukocytes (WBC) or bone marrow cells (BM). Specific incorporation is the ratio of the [^{3}H]thymidine incorporated (count/min) in mitogen-treated cells to that incorporated in control cells. Ranges are given in parentheses.

Patients (No.)	Type of cells cultured*	Specific incorporation in presence of			
		PHA-M† at		PWM† at	
		1 : 10	1 : 100	1 : 10	1 : 100
		IgG myeloma cells			
9	WBC	43.3 (79–31)	2.1 (2.9–1.1)	97 (103–89)	5.1 (5.9–5.0)
	BM	2.1 (3.9–0.8)	1.1 (1.6–0.8)	37 (41–31)	1.4 (1.7–0.8)
		IgA myeloma cells			
3	WBC	51.7 (64–42)	0.8 (1.3–0.6)	72 (89–51)	4.2 (4.8–3.9)
	BM	1.2 (1.7–1.0)	1.0 (1.3–0.8)	23 (29–17)	0.8 (1.7–0.7)
		X-linked agammaglobulinemia cells			
2	WBC	49 (56–42)	0.9 (1.1–0.8)	3.4 (3.7–3.1)	1.1 (1.5–0.6)
	BM	1.4 (1.8–1.0)	1.1 (1.3–0.9)	0.9 (1.3–0.6)	1.2 (1.5–0.9)
		Anemia cells			
7	WBC	57 (68–40)	7.1 (8.4–6.2)	79 (108–64)	2.9 (3.5–2.6)
	BM	1.6 (2.3–0.9)	1.0 (1.7–0.7)	29 (32–23)	1.9 (2.7–1.3)
		Normal cells			
9	WBC	51 (39–68)	3.4 (7.4–2.1)	84 (97–71)	3.7 (4.1–3.2)

* Cells obtained from the sucrose gradient were cultured, 1×10^6 cells per tube for 3 days (*12*); 1 μc of [^{3}H]thymidine was added 16 to 18 hours before the termination of cell culture; † 0.1 ml added to each tube at day 0. Dilutions below 1 : 10 were toxic and those above 1 : 100 gave no responses.

Table 3. The in vitro proliferative responses and the binding of antiserum to immunoglobulin (Anti-Ig) to peripheral leukocytes (WBC) or to bone marrow cells (BM). Cells were fractionated on sucrose gradients (*11*) and then washed and cultured (*12*) in the presence of antiserum to Ig or incubated with ^{125}I-labeled antiserum to Ig (*13*). The specific incorporation is the ratio of counts per minute of cells in the presence of antiserum to Ig or normal goat serum (NGS) over that in their absence. The specific binding is the ratio of ^{125}I (count/min) of cells in the presence of ^{125}I-labeled antiserum to Ig to that in the presence of ^{125}I-labeled NGS. Ranges are given in parentheses.

Patients (No.)	Cells tested	Specific incorporation			Specific binding	
		Anti-IgG	Anti-IgA	NGS	^{125}I-Anti-IgG	^{125}I-Anti-IgA
		IgG myeloma				
9	WBC	2.1 (2.3–1.9)	1.4 (1.7–1.2)	1.8 (2.1–1.6)	19.7 (27.8–9.4)	1.9 (3.4–0.9)
	BM	11.2 (15.3–9.8)	0.9 (1.1–0.6)	1.2 (1.4–1.0)	17.4 (20.2–15.9)	1.2 (1.3–0.8)
		IgA myeloma				
3	WBC	1.3 (1.3)	3.2 (3.7–2.9)	1.1 (1.7–0.8)	1.2 (1.7–1.0)	14.1 (31–8.5)
	BM	1.4 (1.7–1.1)	14.6 (27.1–9.3)	1.3 (1.5–1.1)	1.8 (2.3–1.1)	21.7 (22.5–20.1)
		X-linked agammaglobulinemia				
2	WBC	1.2 (1.5–0.9)	0.7 (0.8–0.6)	1.4 (1.6–1.2)	2.9 (5.7–1.1)	0.5 (0.7–0.3)
	BM	1.7 (1.9–1.5)	1.2 (1.4–1.0)	1.1 (1.8–0.4)	1.4 (1.9–0.9)	1.2 (1.6–0.8)
		Anemia				
7	WBC	5.6 (6.7–5.4)	2.2 (2.3–2.0)	1.3 (1.9–0.9)	11.9 (14.2–8.9)	2.7 (3–1.9)
	BM	1.3 (1.6–1.2)	1.2 (1.5–1.0)	1.6 (1.9–1.4)	12.3 (13.1–11.7)	3.5 (3.9–3.2)
		Normal				
9	WBC	5.8 (6.1–5.4)	1.4 (1.7–1.2)	0.9 (1.2–1.8)	14.7 (17–11.5)	5.1 (5.9–3.8)

PHA. Bone marrow cells of myeloma patients, enriched with plasma cells, gave a stronger response to PWM than controls. Though leukocytes of the agammaglobulinemia patients tested did respond to PHA, both their leukocytes and bone marrow failed to respond to PWM.

Table 3 represents both the in vitro blastogenic response to antiserum to Ig and the binding to [^{125}I]antiserum to Ig by the incubated cells. The myeloma cells bind to and proliferate in the presence of the specific antiserum to Ig. Leukocytes from myeloma patients failed to undergo blastogenesis but showed specific binding. Whereas bone marrow cells from controls could bind to antiserum to IgG, they failed to show proliferative responses in its presence. Leukocytes of controls showed both blastogenesis and binding to antiserum to IgG. Bone marrow and leukocytes of patients with X-linked agammaglobulinemia failed to undergo blastogenesis and to show binding in the presence of antiserums to IgA or IgG.

The foregoing data indicate that bone marrow cells of both controls and patients with multiple myeloma do respond in vitro to PWM but not to PHA. Moreover, bone marrow cells carry Ig receptors as tested by the in vitro proliferative responses or the binding to the specific antiserum to Ig. When the responses of normal bone marrow cells and myeloma bone marrow cells to the antiserum to Ig are compared, it can be seen that in multiple myeloma the number of cells carrying these receptors or the number of receptors per cell is greater than in controls. The failure of normal bone marrow to undergo blastogenesis could not be attributed to a subthreshold quantity of receptors per cell nor to a fewer number of cells carrying these receptors, since quantitatively bone marrow cells did bind to the labeled antiserum to IgG to almost the same extent as leukocytes. More work has to be done to explain this phenomenon. A second unexplained observation is the failure of leukocytes from myeloma patients to respond to the antiserum to Ig. Though we do not have any experimental data, it is possible that these cells are heavily coated with serum Ig and thus the surface receptors are masked and inaccessible to interact with the antiserum to Ig. Bone marrow cells and leukocytes of patients with X-linked agammaglobulinemia failed to respond to both PWM and to antiserum to Ig. On the other hand, leukocytes of these patients did respond in a normal fashion to PHA.

The above findings would indicate that human bone marrow contains cells that behave in vitro in a manner similar to BD cells. These are present in excess in cases of multiple myeloma and are absent in cases of sex-linked agammaglobulinemia. Peripheral blood leukocytes, however, respond to PWM, PHA, and antiserum to Ig, indicating that they contain a mixture of BD and TD cells. From our results and those of others (*6–9*), the response in vitro of lymphoid cells to both PWM and antiserum to Ig may be taken as a criterion for the presence of BD cells among the cells tested.

In the chicken, immunoglobulin synthesis and cells carrying Ig receptors are first detected in the bursa of Fabricius during embryonic life (*6*). In man, though we have demonstrated BD cells and not TD cells in the marrow, we have no direct evidence that the bone marrow stem cell undergoes differentiation into Ig-carrying cells in situ. We cannot exclude from our study the possibility of maturation of the stem cell into bursal-like cells in other organs or the presence of a small number of TD cells in the marrow that we were unable to detect by our technique. Studies of the fetal cells at different stages of development will clarify the matter.

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10. Bone marrow aspirate or peripheral blood was collected in heparin (100 unit/ml) in sterile plastic tubes (Falcon No. 2001) and allowed to settle for 2 hours at 37°C. The plasma cell layer is aspirated and washed in medium 199 containing 10 percent γ-globulin-free pooled human AB serum.
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13. P. J. McConahey and F. J. Dixon, *Int. Arch. Allergy* **29**, 185 (1966). One milligram of monospecific purified goat antiserum to human Ig (Hyland) was conjugated to 1 mc of ^{125}I (International Chemical and Nuclear). The binding of the ^{125}I-labeled antiserum to Ig to the cells was achieved by incubating 1×10^6 cells with 100 μg of the labeled antiserum at 4°C for 2 hours under continuous gentle shaking. Cells were then washed three times and the radioactivity was counted in a gamma counter.
14. All the antiserums to immunoglobulins were tested for monospecificity by immunodiffusion and by their capacity to agglutinate tanned sheep red cells coated with purified immunoglobulins. To test for the specific antibody content of the antiserums to IgA or IgG, they were adsorbed with specific immunoadsorbents according to the method of Avrameas and Ternynack (*15*). The adsorbed antibody was eluted, tested for protein content, and then sterilized by Millipore filtration (0.45 μm pore size) before use.
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L-Leucine: A Neuroactive Substance in Insects

Abstract. *A compound was isolated from the blood of silkworm larvae,* Bombyx mori, *which had been prostrated with DDT; this compound increased the spontaneous discharge in the isolated abdominal nerve cord of the American cockroach,* Periplaneta americana. *The compound was identified as* L-*leucine.*

Sternburg and Kearns have reported that an unknown neuroactive substance is released into the blood of American cockroaches and crayfish during DDT poisoning (*1*, *2*). We now report that a neuroactive substance was isolated from the blood of silkworms, *Bombyx mori*, that had been prostrated with DDT; the compound was identified as L-leucine.

Four thousand fifth-instar larvae of silkworms were treated with a solution of DDT in acetone (approximately 1 mg of DDT per larva) and bled after being kept at 26°C for 12 hours. The blood was dropped from the cut ends of prolegs into a chilled mixture of ethanol and Dry Ice. The collected blood (about 450 g) was deproteinized with chilled ethanol, which was then evaporated at reduced pressure in an atmosphere of carbon dioxide. The residue was fractionated by column chromatography (i) through cellulose powder eluted with a mixture of *n*-butanol, acetic acid, and water (4 : 1 : 5) and (ii) through Sephadex LH-20 eluted with aqueous ethanol. Each fraction was examined by paper chromatography developed with a solvent mixture [*n*-butanol, acetic acid, and water (4 : 1 : 5)] (*3*).

The assay of neuroactivity was carried out at 22° to 26°C by recording the spontaneous discharge in the isolated abdominal nerve cord of the American cockroach, *Periplaneta americana*, immersed in a physiological saline solution in a small chamber (2.5 ml). A small portion of the cord between the fifth and sixth ganglia was kept out of the solution to place on a recording Ag-AgCl electrode (100 μm in diameter) in the air. An indifferent electrode was placed in the solution. The test sample dissolved in a saline solution was applied into the chamber.

A neuroactive fraction contained three substances (R-1, R-2, and R-3), which were positive to ninhydrin and diazotized *p*-nitroaniline and had R_F's of 0.62, 0.58, and 0.48, respectively. Substances R-1 (6 mg) and R-3 (6 mg) were isolated in colorless crytalline state by fractional crystallization. The purified R-3 did not show any neuroactivity, but R-1 was highly active (Fig. 1a). Substance R-1 resembled the substance reported by Sternburg and Kearns (*2*, *4*) with respect to its action on the insect nervous system and to its chromatographic behavior, except for the positive ninhydrin test; but by paper chromatography it was different from some known neuroactive substances such as acetylcholine, dopa, dopamine, adrenaline, noradrenaline, 5-hydroxytryptamine, glutamic acid, and γ-aminobutyric acid.

Substance R-1 decomposed at 250° to 251°C. It did not show any characteristic ultraviolet absorption spectrum. The mass spectrum of R-1 had a small peak at *m/e* (mass/charge) 131, strong ones at 86, 74, 44, and 43 and a moderate one at 57. They could be assigned as the molecular ion (M^+),

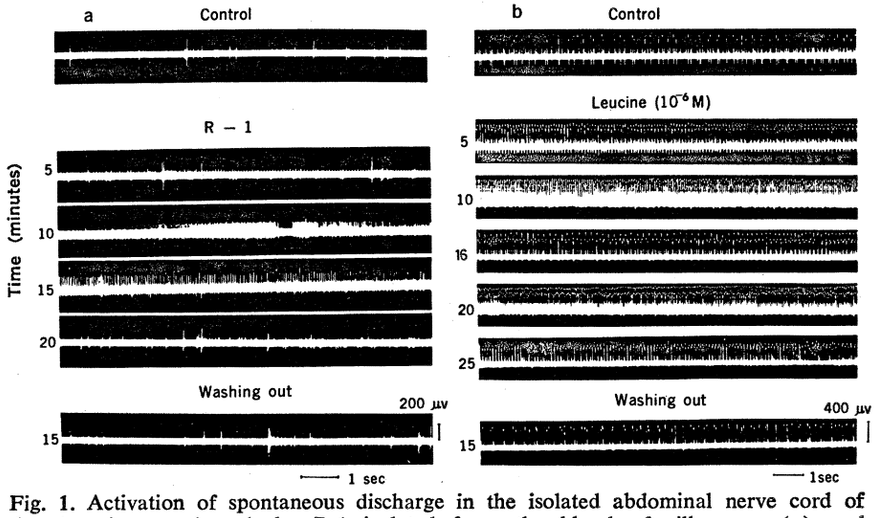

Fig. 1. Activation of spontaneous discharge in the isolated abdominal nerve cord of the American cockroach by R-1 isolated from the blood of silkworms (a) and authentic L-leucine at $10^{-6}M$ (b).