Bone Marrow and Spleen: Dissociation of Immunologic Properties by Cortisone

Abstract. *Bone marrow and spleen cells react differently after treatment with cortisone acetate in vivo. Antibody-forming, hematopoietic, and proliferative responses of spleen cells are reduced, while bone marrow cell responses are not. The stimulation of spleen cells by phytohemagglutinin is abolished, but the response of marrow cells is enhanced. These reactions provide functional markers for the different cells involved in immunologic responses.*

The role of bone marrow cells in immunologic responses is becoming more apparent. Marrow interacts synergistically with thymus cells in the production of antibody in the mouse (*1*), repopulates the thymus after irradiation (*2*), and contributes effector cells of delayed hypersensitivity reactions (*3*). Very little is known, however, about the nature of the specific marrow cell or cells involved in these reactions and about the relation, if any, to other cell types and other functions of the bone marrow. Because cortisone is a known immunosuppressive agent and destroys lymphocytes throughout the body (*4*), we have used it to investigate the cellular role of bone marrow in the immune response.

Adult LAF_1 male mice were given two intraperitoneal injections of 12.5 mg of cortisone acetate, 36 hours apart (*5*). The mice were killed 12 hours after the last injection, and single cell suspensions were made of spleen and bone marrow. The cells were washed twice in spinner-type minimal essential medium (MEM) and assayed for their proliferative and hematopoietic activity, their ability to produce antibody, and their response to the mitotic stimulator phytohemagglutinin (*6*).

Antibody-forming capacity was measured by injecting the spleen cells and sheep erythrocytes (SRBC) (0.1 ml of 20 percent SRBC) intravenously into lethally irradiated (1000 r of ^{60}Co) isologous mice. Each recipient was given an intraperitoneal injection of 0.5 μc of $^{59}FeCl_3$ 24 hours before killing. Six days after transfer, the spleens of the recipient mice were removed, the spleen index was calculated [spleen index = spleen weight (mg) divided by body weight (g)], and each spleen was counted in a well-type gamma scintillation counter for ^{59}Fe uptake. The number of cells producing hemolytic antibody to the SRBC (plaque-forming cells or PFC) was assayed by the Jerne technique (*7*).

Antibody-forming capacity of bone marrow was assayed by transferring the bone marrow cells, normal thymus cells, and SRBC intravenously to lethally irradiated recipients. Four days later, these recipients were given a booster injection (intraperitoneal) of 0.5 ml of 10 percent SRBC. On the eighth day after transfer the recipients were killed, and the spleen index, ^{59}Fe uptake, and PFC were assayed as above.

The phytohemagglutinin responses of spleen and marrow were tested by incubating the cells at 37°C in 5 percent CO_2 in a tissue culture medium containing 20 percent heat-inactivated fetal calf serum in MEM with added glutamine (1 m*M*), penicillin (80 unit/ml), and streptomycin (100 μg/ml). Portions (1 ml) of the cell suspensions were incubated in triplicate, with or without phytohemagglutinin (phytohemagglutinin-M, Difco). DNA synthesis was determined as follows. On the third day of culture [*methyl*-^{3}H]thymidine was added for 5 hours, and the radioactivity of a hyamine digest of cells was measured in a liquid scintillation counter (*8*).

Groups A and B (Table 1) demonstrate that, when equal numbers of cells were compared, cortisone-treated spleen cells were less efficient than normal cells both proliferatively (spleen index) and immunologically (PFC). Since a whole spleen from a cortisone-treated mouse contained an average of only 8 percent as many cells as a spleen from a normal animal (see Table 2), each whole spleen of a cortisone-treated donor had 1/15 of the proliferative, 1/17 of the hematopoietic, and 1/92 of the antibody-forming ability of a whole spleen of a normal donor.

The bone marrow (BM) (Table 1, Groups C, D, and E) showed a different response. Group C, which received cortisone-treated marrow, had both an increased spleen index and an increased ^{59}Fe uptake, but did not show a signifi-

Table 1. Effect of cortisone on proliferative and antibody-forming capacities of bone marrow and spleen. Lymphoid cells were transferred with 0.1 ml of 20 percent SRBC. Groups A and B were killled 6 days after transfer: C, D, and E were boosted with SRBC at 4 days and killed at 8 days. Experimental values give the means with 95 percent confidence limits of the mean. The derived values were calculated correcting the response for the percent of normal donor organ (spleen or femur) used in transfer.

Group	Cells transferred	Recipient mice (No.)	Experimental values			Derived values		
			Recipient* spleen index	^{59}Fe uptake per recipient spleen (count/min)	PFC per† recipient spleen	Spleen index per donor organ	^{59}Fe uptake per donor organ (count/min)	PFC per donor organ
A	11×10^6 Cortisone spleen + SRBC	5	2.00 (1.80–2.21)	1158 (1046–1271)	137 (98–192)	1.27 (1.14–1.41)	758 (685–832)	87 (62–122)
B	11×10^6 Normal spleen + SRBC	6	2.33 (2.08–2.57)	1524 (1076–1972)	959 (768–1197)	19.21 (17.40–21.49)	12705 (8970–16439)	8029 (6440–11011)
C	10×10^6 Cortisone BM 27×10^6 Normal thymus + SRBC	6	6.62 (5.56–7.67)	1927 (1509–2345)	3225 (1598–6512)	8.14 (6.84–9.43)	2370 (1856–2884)	3967 (1966–8010)
D	10×10^6 Normal BM 27×10^6 Normal thymus + SRBC	6	5.46 (4.67–6.26)	1585 (996–2174)	4004 (3073–5217)	8.35 (7.15–9.58)	2425 (1524–3326)	6126 (4701–7982)
E	27×10^6 Normal thymus + SRBC	4	1.83 (1.37–2.29)	139 (18–260)	111 (87–141)			

* Spleen index = [spleen weight (mg) divided by body weight (g)]. † PFC = plaque-forming cells.

cant difference in antibody formation (PFC) when compared to controls which received normal bone marrow (Group D). Because an average cortisone-treated femur contained 80 percent of the cells of an average normal femur (Table 2), the derived values of Table 1 show that cortisone treatment did not significantly affect the proliferative, hematopoietic, and antibody-forming capacities of a whole donor femur.

When normal bone marrow and spleen cells were incubated with phytohemagglutinin (Table 3), the uptake of [*methyl*-^{3}H]thymidine by spleen was stimulated, whereas the uptake by marrow was not. If these cells were taken from cortisone-treated donors, the reverse was true; cortisone treatment abolished the phytohemagglutinin response of spleen cells but enhanced the phytohemagglutinin response of marrow cells.

These results must be considered in light of reports which show that more than one cell type is required for the antibody response of the mouse. Most of the hemolytic antibody to intravenously administered SRBC in the rodent is made in the spleen, and spleen cells can adoptively transfer antibody-forming capabilities to irradiated, immunologically incompetent hosts (*9*). In similar transfer experiments, thymus or bone marrow cells alone cannot make antibodies to SRBC, but combinations of both thymus and marrow cells are immunocompetent (*1*). In these thymus–marrow combinations, the antibody is manufactured by marrow-derived cells while the thymus cells act in a way that is still unknown but which probably involves antigen handling (*10*). Both cell types are radiosensitive (*11*). More recently, it was found that the spleen itself also contains at least two kinds of cells required for the response to SRBC (*12*) and that one of these populations is thymus-dependent (*13*).

Table 3. Phytohemagglutinin response of bone marrow and spleen cells from normal and cortisone-treated mice. Cortisone treatment consisted of intraperitoneal injections of 12.5 mg of cortisone acetate 48 and 12 hours before donor mice were killed. Marrow and spleen cells were cultured in triplicate for 3 days, and 2 μc of [*methyl*-^{3}H]thymidine was added to each tube for 5 hours. Phytohemagglutinin was added as 0.1 ml of a 1 : 5 dilution for marrow and 0.1 ml of a 1 : 10 dilution for spleen.

Cell donor treatment	Cells per culture	Radioactivity in cells receiving		Stimulation by phytohemagglutinin
		No treatment (mean count/min ± S.E.)	Phytohemagglutinin (mean count/min ± S.E.)	
		Bone marrow		
None	4×10^6	993 ± 148	1073 ± 63	None
Cortisone	4×10^6	981 ± 43	2535 ± 501	2.6 ×
		Spleen		
None	6×10^6	640 ± 66	2899 ± 374	4.5 ×
Cortisone	6×10^6	19 ± 4	25 ± 10	None

The high susceptibility of the spleen to cortisone, as well as the fact that cortisone is far more immunosuppressive if given early rather than late in the immune response (*5*), indicates that cortisone interferes with antibody formation in the spleen at some early stage which possibly involves thymus-derived cells. The lack of stimulation of cortisone-treated spleen cells by phytohemagglutinin would be in accord with this concept because most splenic cells responsive to phytohemagglutinin are thymus-dependent (*14*).

The finding that cortisone-treated bone marrow retains its immunocompetence (when combined with normal thymus) indicates that the precursor of the antibody-forming cell is unaffected by cortisone (at least when this cell is in the marrow). The increased marrow response to phytohemagglutinin after cortisone treatment may reflect an enrichment of the small number of phytohemagglutinin-responsive cells in normal marrow or the removal of inhibiting or competing factors.

Immunocompetence and responsiveness to phytohemagglutinin of spleen are depressed by neonatal thymectomy (*14, 15*) and by cortisone treatment. On the other hand, neither cortisone nor neonatal thymectomy (*16*) impair the immunocompetence of marrow. Responsiveness to phytohemagglutinin of marrow is also not depressed by cortisone, but the effect of neonatal thymectomy is not known. These similarities suggest that thymus-derived cells are very sensitive to cortisone. The absence of cortisone-sensitive cells in the marrow may be due to the lack of thymus-derived cells or to unique environmental influences.

Large doses of corticosteroids depress hematopoiesis in the mouse, although the relative roles of spleen and bone marrow in this situation have not been studied (*17*). Our data show that marrow transferred from cortisone-treated donors has unimpaired proliferative and hematopoietic abilities. The marked depression of these abilities in the cortisone-treated spleen may indicate that hematopoietic cells in spleen are in a different stage of maturation compared to hematopoietic cells in marrow. An alernative possibility is that impairment of hematopoiesis in the spleen is secondary to the massive collapse of this organ after destruction of splenic lymphoid tissue by cortisone. The differential susceptibilities of marrow and thymus cells to cortisone will permit further dissection and analysis of the cellular events in antibody production.

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Table 2. Effect of cortisone acetate on cellularity of mouse bone marrow and spleen. Cortisone acetate (12.5 mg) was injected (intraperitoneally) 48 and 12 hours before animals were killed.

	No. of organs measured	Nucleated cells ($\times 10^6$) (mean*)	Percent
	Femur		
Normal	16	15.3 (13.1–17.5)	100
Cortisone treated	6	12.3 (10.3–14.4)	80.4
	Spleen		
Normal	17	91.7 (81.8–101.6)	100
Cortisone treated	9	7.2 (5.4–9.1)	7.9

* $P \leq 5$.

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Human Auditory Evoked Potentials: Possible Brain Stem Components Detected on the Scalp

Abstract. *Auditory potentials recorded from the vertex of humans by a modified averaging technique have very short latencies and are probably generated by brain stem structures located at a considerable distance from the recording point. The evoked waves, which show considerable detail and consistency within and across subjects, may be clinically useful in evaluating subcortical function.*

The use of averaging techniques in the study of the human auditory system has been limited to auditory nerve potentials recorded from the ear canal (*1*), and to waves, recorded from the scalp, which may reflect cortical activity (*2*). Recently, waves with peak latencies as short as 7.6 msec have been recorded from the scalp (*3*). We now report a method which can record from the scalp electrical activity within the interval which the previous methods have not studied. Thus, a combination of our method with the ear canal method (*1*) should allow study of the electrical activity of most parts of the auditory system, including brain stem components, in intact, unanesthetized humans. Such recordings could be used to determine the neural level of dysfunction in clinical conditions such as severe brain damage and space-occupying lesions, since it is likely that wave components can be related to specific neural structures, as is the case in the cat (*4*), bat (*5*), and chinchilla (*6*). Our recordings show that in man, as in cats (*4*), averaging techniques can now be used to detect neural signals at such distances that the electrical activity from any brain location within the skull should be detectable at the scalp, given a satisfactory method of synchronizing the activity with the averager.

Three adult male subjects were studied at rest in an electrically grounded metal enclosure, in a seated position with the head supported and the neck muscles relaxed. The subject was grounded by the earlobe or wrist. The signal was led from electroencephalograph (EEG) disk electrodes to, in succession, a Grass P9 preamplifier, a high-pass filter (bandpass of system approximately 300 hz to 2 khz at 6-db points), a Tektronix 3A3 amplifier in a 565 oscilloscope, and a Princeton Applied Research TDH-9 waveform eductor (a 100-channel capacitor-storage "averager"). The TDH-9 was used on-line in a manner which minimized the nonlinear summation of the instrument by a sweep speed of 100 microseconds per point and a 100-second capacitor time-constant; these settings gave, with maximal input voltages and 2000 repetitions, signal outputs of 50 to 100 mv peak-to-peak, approaching the performance limit of the TDH-9. A Tektronix 162 waveform generator, carefully set at a frequency which canceled 60-hz power-line interference (*4*), triggered the TDH-9 at about 16 hz and, after a delay, an Exact 303 square-wave generator which in turn delivered a 0.5-volt, 0.1-msec pulse to an Audiovox 9C earphone attached by a Y tube to stethoscope earpieces for binaural stimulation. Click intensity, measured in a quiet room by using a power attenuator box with properly matched impedances, was never greater than 75 db above the subject's subjectively determined threshold for this stimulus. The click waveform (Fig. 1A) was transduced to the oscilloscope by a 12.5-mm condenser microphone with a Bruel and Kjaer Type 2203 sound level meter through a 2-cm^3 coupling chamber.

Recordings were made between the vertex and the lateral-posterior neck; similar results were obtained with the earlobe or chin as reference point. For all recordings 2000 clicks were delivered. Auditory responses (Fig. 1, B–D) showed a series of waves between 2 and 7 msec after the arrival of the stimulus at the ear, which were surprisingly consistent within and between subjects. These waves all have shorter latencies and apparently greater consistency than those of the earliest potentials recorded by Mendel and Goldstein (*3*), which in turn are reported to be more stable than waves with latencies of 50 msec or greater. Control records (Fig. 2A) taken with the ears plugged showed an absence of electrical artifact beyond the first fraction of a millisecond. There was no reversal of the polarity of the evoked potential with stimulus reversal, indicating that no part of the cochlear microphonic was detected. The first detectable wave had a magnitude of about 100 nanovolts, approached the level of run-to-run variation in the base line (see Fig. 1C; compare with Fig. 1, B

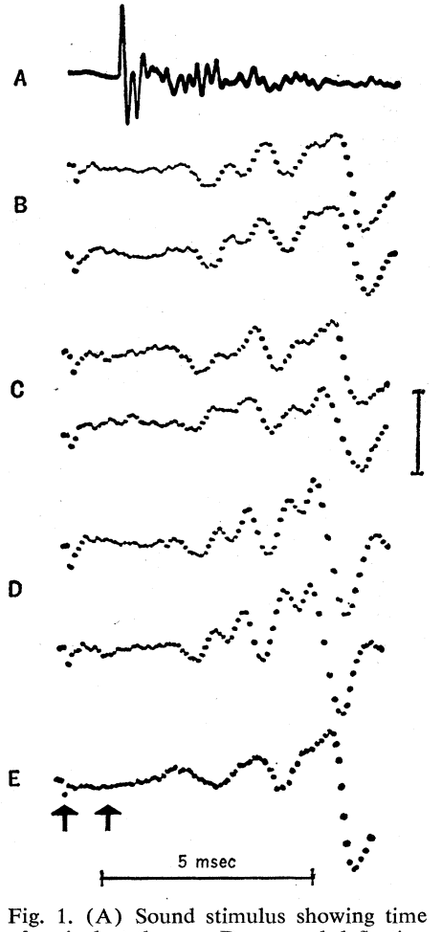

Fig. 1. (A) Sound stimulus showing time of arrival at the ear. Downward deflection indicates compression. (B–D) Two recordings from each of three subjects, vertex-neck, approximately 75-db sensation level. (E) Recording vertex-neck from subject in D, 65-db sensation level. Vertical calibrations: A, 14 μbar; B–E, 0.5 μv. In B–E and in Fig. 2, upward deflection indicates positivity at the first electrode; earphones were energized at the first arrow; sound wave arrived at the ear at the second arrow.