To the Editor:

The article by Benestad et al.1 published in BLOOD states that murine bone marrow functions are not regulated by neuronal mechanisms. I am surprised by this drastic and final conclusion. Even the title, “No Neuronal Regulation of Murine Bone Marrow Function,” sounds more suitable for a popular press article.

Actually, there are a number of observations that have been disregarded by the investigators and some most recent findings that should be taken in consideration.

(1) A recent publication that has been ignored by the investigators shows that surgical denervation decreases femoral cellularity as well as progenitor cells while mobilizing these cells in the peripheral blood of splenectomized mice. In nonsplenectomized animals, these changes were quickly cleared.2 In addition, Benestad et al.1 did find a decreased bone marrow cellularity in chemical sympathectomized mice.

(2) The investigators argue that our results with adrenergic agonists and antagonists that suggest the presence of a catecholaminergic regulation of haemopoiesis are due to nonspecific effects. This seems rather superficial, because, besides the findings quoted, we demonstrated also that (a) bone marrow pre-B cells do express α1B-adrenergic receptors,3,5 (b) that in vitro norepinephrine (NE) and other adrenergic agonists can inhibit myelopoiesis and rescue bone marrow progenitors from the toxic effect of cytotoxic drugs, and that the α1-adrenergic antagonist prazosin neutralizes these effects at concentrations of $10^{-10}$ to $10^{-12}$ mol/L.3,6 (c) NE protects 77% of mice injected with a supralethal dose of carboplatin (200 mg/kg) and prazosin abolishes the protection. A time-course study showed that this effect was exerted directly on hematopoietic progenitors in the bone marrow.6

Most recently, we demonstrated that murine bone marrow contains substantial amounts of catecholamines. NE and dopamine (DA) showed a daily rhythmicity, with peak values during the night. The rhythm was disrupted by chemical sympathectomy, whereas epinephrine (E) did not show any rhythmicity or sensitivity to 6-hydroxydopamine. High and low values of NE and DA were associated with high and low values of their metabolites, which indicated a rhythmic catecholamine release. NE but not DA and E were positively associated with the proportion of cells in the G2/M and S phases of the cell cycle. Moreover, NE and DA were found in both short-term and long-term bone marrow cultures as well as in human or murine B-lymphoid cell lines. These findings indicate that endogeneous catecholamines in the bone marrow have both neural and cellular origin.5

In conclusion, the negative results obtained by Benestad et al.1 might well reflect a long-term adaptative response of bone marrow cells catecholamines, but in no case can rule out a neuronal influence on hematopoiesis. Finally, as the investigators also beautifully show, bone marrow is richly innervated by afferent adrenergic fibers. Should we argue that such rich innervation is useless?

Georges J.M. Maestroni
Istituto Cantonale di Patologia
Center for Experimental Pathology
Locarno, Switzerland

REFERENCES


Neural Regulation of Bone Marrow

To the Editor:

Benestad et al.1 recently published a paper in BLOOD that presented data showing no effect of interfering with neural input to the bone marrow in mice. Specifically, they found no effect of cutting the sciatic nerve or femoral nerve, of neonatal sympathectomy, or of electrical stimulation of the nerve. In addition, they found no effect on blood flow to the marrow of any of these procedures but note that the overlying muscle vasculature did respond. Thus, they concluded that they could not ascribe any function to the innervation of the marrow and that this finding “supports the physiologic relevance of ex vivo experiments on bone marrow.”

There are a number of methodological issues arising in the experiments reported in Benestad et al.1 that may have led to the data described that are contrary to those we have reported.2 We showed a marked effect of femoral denervation on cell egress and cellular retention in the femoral bone marrow as well as on the movement of immature and mature cells between the marrow and peripheral circulation. Perhaps significantly, we have identified the followed methodological differences between the reports.

(1) The sciatic nerve was cut at the level of the sciatic notch by Benestad et al.1 However, we found that cutting the nerve at these low levels produced no effect on the femoral marrow. Although the investigators found loss of innervation, as demonstrated by glyoxylic acid staining, on blood vessels near the tibia, it is still possible that crucial nerve projections in the other nerves and branches that project down the leg to the tibial region, including the subbranches of the sciatic and femoral nerve, were left intact. In tracer studies, we injected horseradish peroxidase into the femur and obtained stained profiles in the obturator and obdurator nerves, among other nerves projecting down the leg (unpublished data). The sciatic nerve is complex and composed of fibres emanating from at least 5 nerve roots of the spinal cord. In our experiments, we cut all of these nerve roots at their exit from the spine.
(2) In the electrical stimulation studies, the investigators first cut the sciatic nerves and then stimulated the cut end on one side of the animal. They used chloral hydrate and pentobarbitone anaesthesia throughout these experiments. If the nerve input is indeed important, it is difficult to imagine that cutting the nerve and then stimulating it will produce a clear result as much of the effect of nerve cutting is unlikely to be recovered by nonspecific stimulation of all the fibers entering the system. Furthermore, we have also found that pentobarbitone anaesthetic has the same effect as cutting the nerve, so that recovery from the nerve cut, or any significant effect of nerve stimulation, is highly unlikely in these experiments.

(3) The investigators used neonatal sympathectomy to investigate the role of adrenergic input to the marrow. If, as we and others have proposed,9 the nervous system has an executive role in coordinating and regulating host defence, then permanent deletion of a pathway of control (particularly in early development of the control system) will not show the significance of that pathway to normal, adult physiology. Of course, the response to a challenge may well expose this missing coordination pathway. We found some recovery from the effects of whole nerve denervation after 14 days and during sympathetic blockade in adults. Given this recovery from denervation in adult animals, it is unlikely that neonatal deletion will show significant effects. Benestad et al10 acknowledge this possibility in their discussion.

An interesting aspect of the data is the finding that denervation did not affect the vascular volume of the bone, suggesting that the innervation is not involved in vasomotor control as in other tissues such as muscle. Thus, one must ask the question of what is the role for the innervation to the bone marrow? Yamazaki and Allen4 showed clear synaptic connections between nerve terminals and perivascular/stromal cells. It is inconceivable that such a relationship is without function, and our experiments have demonstrated that this innervation probably controls the blood-marrow interface, presumably through control of the perivascular cells. Furthermore, our data implicate adrenergic input in this control and suggest that other transmitters are involved in the retention of cells within the marrow itself. This pattern of control through accessory and stromal cells has been found in other lymphoid tissues, including the thymus and lymph nodes.

In conclusion, aspects of the methodology used in the study by Benestad et al10 may have led to the negative data produced. With a complicated system such as host defence and immunity, a reductionist approach (ie, ex vivo or in vitro) has many advantages in the dissection of the system. However, to exclude the influence of other body systems, notably the neuroendocrine system that has been shown to have major influences on both immunity and host defence, denies an integration into the physiology of the whole body.

J.A. Miyan
C.S. Broome
A.D. Whetton
Department of Biomolecular Sciences
UMIST
Manchester, UK

REFERENCES

Response: Nerves to Murine Bone Marrow: Roles in Cell Production or Cell Release?

To Dr Maestroni’s specific points, we make the following comments.
(1) The recent publication that he claims was ignored by us (Afan et al10) is indeed interesting. It was received by the library of our National Hospital 1 month after we submitted our manuscript2 to BLOOD, so we could not discuss its findings. However, it is commented on by us now (see below).

(2) We wrote, “It is possible that effects, which may be both nonspecific and not related to the innervation of bone marrow, may take place after treatment of adult, nonadrenalectomized animals with 6-OH-DA. The same kind of objection could be raised against the interpretation of the prazosin results.” We cannot see that any of the data or arguments in Dr Maestroni’s letter invalidate this statement. His claim that “A time-course study showed that this effect was exerted directly on hematopoietic progenitors in the bone marrow” is curious. In the study cited, a response was first recorded 6 hours after initiation of treatment of the mice, a time period long enough for indirect effects to take place, such as induced secretion of cytokines or other agents that might be the ultimate effectors. In fact, the Discussion of the quoted report also contains the following: “This finding suggests that the mechanism of the norepinephrine-induced rescue is indirect, perhaps acting via production of cytokines . . . More comprehensive studies are needed to elucidate norepinephrine’s mechanism of action. . . .”

(3) Although interesting, the summary of Dr Maestroni et al’s data on bone marrow catecholamines does not convince us about a functional role for the bone marrow innervation. It is impossible to judge the validity of these data without access to the full report, which is unpublished. It is puzzling that, on the one hand, norepinephrine can inhibit myelopoiesis, but, on the other hand, showed a positive association with the proportion of bone marrow cells in non-G1/G0 phases of the cell cycle. However, of greater relevance may be their claim that endogenous catecholamines in the bone marrow may have a nonneural origin, thus opening for the possibility that adrenergic effects might take place without the involvement of bone marrow nerves. To the points raised by Miyan et al, we make the following comments.

(1) The points raised about surgical denervation miss the target. Like Miyan et al, we are aware that nerve section at the level of the sciatic notch will not effectively denervate femoral marrow. For this reason, we used the tibia, not the femur, for all these experiments. We checked by mononamine histochemistry the extent of denervation, not only by staining small vessels close to the tibia, but also by examination of marrow plugs expelled from the tibia, and included a statement about this in the report: “Two to 3 days after sciatic nerve section, this system of nerve fibers was either completely absent or reduced to occasional patches.”

(2) Miyan et al suggest that the sympathetic effects on bone marrow are prevented (A) by prazosin and (B) by nonspecific electrical stimulation of all the axons in the nerve. Although none of these hypotheses can explain our results after nerve section, either of them could explain our results after nerve stimulation. In any case, smooth muscles in other tissues do contract in response to nerve stimulation in the presence of prazosin,9 and denervated marrow vasculature relaxed in response to a humoral signal.9 Effects of nerve stimulation were shown, as mentioned in our report, by the skeletal muscular contraction evoked by a test stimulus,
To the Editor:

The recent report of Storek et al. on the high risk of chronic graft-versus-host disease (cGVHD) associated with allogeneic peripheral blood stem cell transplantation (PBSCT) has prompted us to present data that support their findings and add to their observations on acute graft-versus-host disease (aGVHD) and late infections.

Before a neuromuscular blocking agent was applied, and by the contractile response of muscular blood vessels in the electrically stimulated limb.

(3) The possible effects of surgical denervation were searched for 1 to 7 days after the operation, so here at least there was not much time for adaptation. With the neonatally performed chemical sympathectomy, which we consider less specific, the situation is different. We discussed the possibility of nerve regeneration (which was not found in the iris examinations) and compensation by the adrenal glands and other hormonal systems (which was not very likely, because we found no qualitatively different effects of sympathectomy or surgical denervation between mice with and without adrenal glands).

We did not measure “the vascular volume of the bone,” but rather the blood flow to the bone marrow. Considering the histological data published by others, we too had anticipated a constrictive response of bone marrow vessels to electrical stimulation, even though we would not go so far as Miyan et al and claim that “It is inconceivable that such a relationship is without function” (see below). However, blood vessels to leg muscle contracted; marrow vessels did not. Furthermore, as mentioned in our report, this finding is consistent with previous findings with either surgically denervated or chemically sympathectomized rats. Similarly, electrical stimulation of the sympathetic trunk in the rat decreased blood flow to the hind limb skin and muscle, but not to the bone marrow, as we also mentioned in our report.

Miyan et al suggest that denervation lifts a “control on the bone-marrow interface, presumably through control of the perivascular cells.” However, taken at face value, their data showing a loss of approximately 50% of femoral progenitor cells in the denervated limbs and 100% increase in intact, contralateral marrow of splenectomized mice on day 4 after surgery do not make sense to us. According to our own unpublished measurements, which agree well with those of others, about 12% of the total mouse bone marrow is present in a denervated hind limb. Presumably, the intact contralateral femoral marrow is representative of the remaining, innervated 88%. Thus, the recorded progenitor cell loss from the denervated marrow cannot make up for the gain in the innervated marrow. Moreover, their chemical sympathectomy of adult mice had no effect on the number of colony-forming cells in the femur on day 4.

Being physiologists, we would certainly not “exclude the influence of other body systems, notably the neuroendocrine system” on bone marrow function or deny “an integration into the physiology of the whole body.” Our main point is that, by paired comparisons between surgically denervated and sham denervated mouse hind limbs, we could not substantiate any neuronal effects; we did not examine most of the endocrine system.

Nerve effects on bone marrow found by others do not fit into a consistent picture. For example, most published results indicate that transmitters stimulate cell formation or cell release, whereas Maestroni et al claim that adrenergic agents inhibit myelopoiesis and Miyan et al claim that nerves promote cellular retention in the femoral bone marrow. On this background it is important to validate all procedures, sham as well as test ones, and with positive results of the experimental procedures to minimize the possibilities that the results may be due to, or influenced by, for example, a stress response (e.g., corticosterone analyses) or endotoxin exposure.

All publications we are aware of in this field point to a functional role of the bone marrow innervation, as referred to and discussed in our report. There are nerve fibers in the bone marrow containing transmitters for which bone marrow stromal and parenchymal cells possess receptors, and such transmitters have definite functional effects on the relevant cells in vitro. Even then, with all odds pointing to a functional role for the innervation, we could find no significant differences between innervated (and in some cases electrically stimulated) and denervated contralateral bone marrow in our mice, concerning either cell generation or cell mobilization. This was a surprise. In such a situation it may be prudent to consider what Prof Lewis Wolpert writes in his book The Unnatural Nature of Science: “…many of the misunderstandings about the nature of science might be corrected once it is realized just how ‘unnatural’ science is. I will argue that science involves a special mode of thought and is unnatural for two main reasons…Firstly, the world just is not constructed on a commonsensical basis. This means that ‘natural’ thinking—ordinary, day-to-day common sense—will never give an understanding about the nature of science. Scientific ideas are, with rare exceptions, counter-intuitive: they cannot be acquired by simple inspection of phenomena and are often outside everyday experience. Secondly, doing science requires a conscious awareness of the pitfalls of ‘natural’ thinking. For common sense is prone to error when applied to problems requiring rigorous and quantitative thinking: lay theories are highly unreliable…. For example, the creationist view in the middle of the nineteenth century held that species were fixed and all the animals were made perfectly adapted to their environment. But this was clearly not true of some animals: some ducks with webbed feet did not swim and why should blind animals that lived in caves have eyes?…”

H.B. Benestad
P.O. Iversen
A. Njå
Department of Physiology
Institute of Basic Medical Sciences
University of Oslo
Oslo, Norway

REFERENCES

High Risk of Chronic Graft-Versus-Host Disease in Unmanipulated Allogeneic Peripheral Blood Stem Cell Transplantation

To the Editor:

Between March 1993 and May 1996, 31 patients received allogeneic bone marrow transplantation (BMT), whereas 25 patients received PBSCT between June 1996 and November 1997, as primary and unmanipulated transplantsations. Except for 2 patients (1 with aplastic anemia and 1 with paroxysmal nocturnal hemoglobinuria), all of the recipients were suffering from hematological malignancies. All donors were HLA-identical siblings. Demographic and clinical characteristics...
Neural Regulation of Bone Marrow

J.A. Miyan, C.S. Broome and A.D. Whetton