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Invited Review

Is hematopoiesis under the influence of neural and neuroendocrine mechanisms?

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Summary. It is well recognized that the immune response is under the influence of a variety of neural or neuroendocrine mechanisms. Much less studied is the possible influence of these mechanisms on hematopoiesis. Here I review the existing evidence about a neural and/or neuroendocrine regulation of hematopoiesis. The physiology of the blood forming system seems to be controlled at three levels, i.e. at the cellular level by the bone marrow stroma, at the humoral level by hematopoietic cytokines and finally by catecholamines and neuroendocrine factors. Bone marrow catecholamines originate from sympathetic nerve fibers and from hematopoietic cells directly. Catecholamines of neural origin show a circadian rhythmicity. Adrenoceptors present on bone marrow cells include the α1-subtype which seems to mediate the catecholaminergic control of hematopoiesis. Neuroendocrine factors including substance P, neurokinin-A and the pineal hormone melatonin might also influence hematopoiesis by affecting hematopoietic cytokines. In particular, melatonin seems to affect hematopoiesis via the induction in bone marrow T-helper cells of two novel opioid cytokines. A complete understanding of the neural and neuroendocrine regulation of hematopoiesis might provide new conceptual and therapeutic perspectives in a variety of hematopoietic and immune diseases.

Key words: Hematopoiesis, Adrenergic receptors, Catecholamines, Melatonin, Cytokines

Introduction

The existence of a common messenger/receptor network between the central nervous system and the immune system is today beyond any doubt. Cytokines which play a crucial role in the immune response may also be produced in the central nervous system and affect neuroendocrine functions. Neurohormones may, in turn, be synthesized by immunocompetent cells and affect immune functions (Ader et al., 1995). On the other hand, almost all cytokines have been shown to affect the blood forming system. The link between the nervous and neuroendocrine systems and hematopoiesis seems thus straightforward. However, the experimental evidence for any neural or neuroendocrine/hematopoietic relationship is scant. Hematopoiesis depends on a highly complex series of events in which a small population of stem cells needs to generate large populations of maturing cells (Galindez and Aggio, 1997). The diverse differentiative and proliferative events as well as entry of mature cells into the circulation and their selective localization in appropriate tissues require a sophisticated regulatory control. Such control may well include neural or neuroendocrine mechanisms. Lymphoid tissues including the bone marrow are directly innervated (Felten, 1993). Neuropeptides and/or neurotransmitters have been reported to influence hematopoiesis via induction of hematopoietic cytokines (Rameshwar et al., 1994; Ader et al., 1995). Consistent with the concept of a neuroendocrine immuno-hematopoietic network, hormonal and neurotransmitters receptors have been described in a variety of immuno-hematopoietic cells (Feldman et al., 1987; Malec and Nowak, 1988; Spengler et al., 1990; Ader et al., 1995). Very little is known about the presence of such receptors in neoplasias of lymphoid tissues. However, the most widely studied molecule on the surface of human leukemic cells, i.e. the common acute lymphoblastic leukemia antigen (CALLA, CD10) has been recently found to be a neutral endopeptidase (E.C.3.4.24.11, enkephalinase) which may suggest an unknown hematopoietic function for enkephalin-containing peptides (LeBien and McCormack, 1989; Delikat et al., 1994). Consistently, it has been recently reported that enkephalins may influence long-term bone marrow cultures (Krizanac-Bengez et al., 1994).

Beside lymphocytes and macrophages which are involved in antigen-specific immune responses, neutrophils constitute the first defence line in case of infectious events and eosinophils have recently appeared to exert a cytotoxic action against tumor cells. Granulocytes are

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short-lived cells with a renewing-time of about 24 hours. Alterations of myelopoietic function are thus mirrored immediately in altered blood counts as occurs during acute infections. Relevant to the topic of this review, I am not aware of any study about a possible neural or neuroendocrine (stress) influence on myelopoiesis either in physiological or in pathological situations (infections).

Catecholamines and hematopoiesis

We have shown that chemical sympathectomy by 6-hydroxydopamine or administration of the α1-adrenergic antagonist prazosin enhance myelopoiesis and exert an inhibitory effect on lymphopoiesis (Maestroni et al., 1992; Maestroni and Conti, 1994b). These results suggested that the function of the blood-forming system is under a sympathetic nervous regulation. Such regulation might be exerted directly on bone marrow cells or indirectly via other mechanisms. To verify this, we investigated whether adrenergic agonists could influence hematopoietic functions in vitro. As a matter of fact, we found that when added directly in bone marrow cultures, adrenergic agonists proved to inhibit the number of GM-CFU (Maestroni and Conti, 1994a). Also, the α1-selective adrenergic agonist methoxamine and to a much lesser extent the α2-agonist clonidine proved to exert an inhibitory action when added in the GM-CFU assay (Maestroni and Conti, 1994a). The relative potency of these adrenergic agonists in inhibiting the number of GM-CFU appeared to be norepinephrine > methoxamine > clonidine (Maestroni and Conti, 1994a). In other experiments, α-adrenergic antagonists such as prazosin, phentolamine or yohimbine were compared for their ability to counteract norepinephrine and the relative order of potency was prazosin ≥ phentolamine > yohimbine (Maestroni and Conti, 1994a). These results were consistent with the presence of α1-adrenergic receptors (α1-ARs) on bone marrow cells. As a matter of fact, a computer-assisted non-linear regression analysis of the isotherm binding of ^{3}H-prazosin revealed the presence of two saturable and specific binding sites (Kd high: 0.98±0.32 nM; Kd low: 55.9±8.2 nM). Competition experiments performed with a variety of α-adrenergic agonists and antagonists gave IC50 and Kd values which were compatible with the presence of a high affinity α1B-AR on bone marrow cells (Maestroni and Conti, 1994a). The remaining site was of less clear characterization and the results obtained were compatible with a low affinity α1-AR. Separation of bone marrow cells by counterflow centrifugal elutriation resulted in separation of the two ARs, with the α1B-AR being partially eluted in a lymphoid fraction containing no blasts and no assayable GM-CFU (Maestroni and Conti, 1994a). Further studies showed that the high affinity, α1-AR is present on loosely adherent Mac1-B220^{+}sIgM^{-} (pre-B) cells. Conversely, the low affinity α1-AR seems to be present on Mac1^{+}B220^{-} cells (Togni and Maestroni, 1996). We also showed that norepinephrine administration in mice rescued hematopoiesis from a lethal dose of the non cell-cycle specific chemotherapeutic agent carboplatin. Protection of granulocyte/macrophage colony-forming units (GM-CFU) was already apparent a few hours after carboplatin and norepinephrine treatment. On day 3, the hematopoietic rescue was reflected by higher leukocyte and platelet counts. At its most effective dose (3 mg/kg, s.c.), norepinephrine protected 77% of the mice injected i.v. with 200 mg/kg of carboplatin (LD 100: 170 mg/kg). The simultaneous administration of the α1-AR antagonist prazosin brought the percent of surviving mice down to 30% indicating that α1-ARs mediated most of the norepinephrine-induced hematopoietic rescue (Maestroni et al., 1997b). Consistently, prazosin administration also reduced blood counts and GM-CFU. In vitro, norepinephrine (1μM) rescued GM-CFU in BM cells and this effect was counteracted by low concentrations (0.1nM-10nM) of prazosin (Maestroni et al., 1997b). More recently, we demonstrated that murine bone marrow contains a substantial amount of catecholamines. Norepinephrine (NE) and dopamine (DA) showed a circadian rhythmicity with peak values during the night. The rhythm was disrupted by chemical sympathectomy while epinephrine (E) did not show any rhythmicity or sensitivity to 6-hydroxydopamine. NE but not DA and E was positively associated with the proportion of cells in the G2/M and S phases of the cell cycle (Maestroni, 1997). Moreover, NE and DA were found in both short-term and long-term bone marrow cultures. These findings indicate that endogenous catecholamines in the bone marrow have both neural and cellular origin (Maestroni et al., 1997a). As the NE-induced inhibition of hematopoiesis may presumably take a few hours to reach its maximum, we are tempted to speculate that NE is involved in the synchronization of the circadian periodicity of hematopoiesis. Although further studies are clearly needed to clarify this point, it is interesting to note that the hematopoietic activity in rodents and humans presents opposite acrophases which are synchronized with the rest-activity rhythm and hence with the rhythm of sympathetic activity (Aardal and Laerum, 1983; Haus et al., 1983; Smaaland et al., 1991).

The fact that bone marrow cells seem to be able to produce catecholamines is also indicative for a physiological role of these substances in hematopoiesis. The cell type which may produce cathecolamines are unknown. It has been recently reported that catecholamines and their metabolites are present in single lymphocytes and extracts of T- and B-cell clones and down-regulate lymphocyte funtion via an autocrine loop (Bergquist et al., 1994). Therefore, bone marrow cathecolamines might derive, in part, from bone marrow lymphocytes or from their precursors. Both for lymphocyte function and hematopoiesis the role of catecholamines seems to be inhibitory (Maestroni et al., 1992; Bergquist et al., 1994; Maestroni and Conti, 1994a,b). To mention just one recent finding, in the case of hematopoiesis, this inhibition might be exploited in

modulating the bone marrow sensitivity to myelotoxic anti-cancer drugs (Maestroni et al., 1997b).

These results emphasize the importance of the functional role of catecholamines in hematopoiesis. Much work is required to further examine this aspect of hematopoietic regulation.

Neuroendocrine influence

The retino-pineal system functions as a resetting system which synchronizes the organism with the photoperiod (Yu et al., 1993). The synchronizing signal is constituted by the indoleamine melatonin which is synthesized and released during the night hours in all species upon activation of pineal ß1 and α1 adrenoceptors (Deguchi and Axelrod, 1973; Yu et al., 1993). In our studies on the immunoregulatory role of melatonin, (Maestroni, 1993) we found that this pineal hormone may rescue hematopoiesis in mice transplanted with Lewis Lung Carcinoma (LLC) and treated with cancer chemotherapeutic compounds (Maestroni et al., 1994a,b). The hematopoietic protection involved the release of granulocyte/macrophage colony-stimulating factor (GM-CSF) from bone marrow stroma upon stimulation by a Th cell factor induced by melatonin (Maestroni et al., 1994a). This factor was immunologically and biologically indistinguishable from interleukin-4 (IL-4) (Maestroni et al., 1996). Nevertheless, further investigations aimed at verifying the melatonin-IL-4 connection failed to confirm this finding. Instead, we found that this Th cell factor was constituted by 2 cytokines of 15 and 67 kDa MW with the common opioid sequence (Tyr-Gly-Gly-Phe) at their amino terminal and a carboxyl-terminal extension which was recognized by both anti-IL-4 and anti-dynorphin B antibodies (Maestroni et al., 1996). Both activated lymph node Th cells and bone marrow Th cells released these opioid cytokines which were named melatonin-induced-opioids (MIO) (Maestroni et al., 1996). Due to their size and unusual immunological characterization, the MIO might represent novel opioid cytokines. The lower molecular weight MIO (MIO-15) seems to mediate both the immunological and hematopoietic effects of melatonin (Maestroni et al., 1996). This finding is consistent with our previous result concerning the ability of the kappa-opioid antagonist dynorphin to mimick the effects of melatonin (Maestroni and Conti, 1989). Interesting enough, in contrast with peripheral Th cells, bone marrow Th cells do not seem to require any antigenic activation to respond to melatonin. This may reflect an inherent difference of bone marrow Th cells from peripheral Th cells and a physiological requirement for sustained melatonin regulation of hematopoiesis. A finding which may support this is that endogenous melatonin may stimulate propiomelanocortin gene expression in rat bone marrow (Ways and Gupta, 1995). This finding is germane to our studies on the hematopoietic action of the melatonin-MIO network (Maestroni et al., 1994a,b, 1996). Most recently, we performed experiments in which we compared the ability of melatonin to protect hematopoiesis in LLC-bearing mice and in tumor-free normal mice treated with the cytotoxic drug cyclophosphamide. This experiment was suggested by the fact that melatonin added in GM-CFU cultures could directly enhance the number of GM-CFU but only in presence of suboptimal concentration of colony stimulating factors (CSF), i.e. in presence of activated bone marrow adherent cells (Maestroni et al., 1994a,b). In addition, LLC is known to produce CSF and exert myelopoietic activity in vivo (Young et al., 1988). Melatonin did not exert any hematopoietic protection in tumor-free mice. Rather, the myelotoxicity of cyclophosphamide was increased by melatonin treatment. However, in both tumor-free and LLC-bearing mice the effect of melatonin was neutralized by naltrexone which suggested the involvement of MIO (Maestroni and Conti, 1997). We found that this dual effect of melatonin seems to depend on the affinity state of a kappa-opioid receptor expressed by bone marrow stromal cells. The presence of GM-CSF seems to increase the affinity of such receptor allowing the MIO to exert the rescue effect. However, when the receptor is in the low affinity state the MIO seems to increase the chemotherapy-induced myelotoxicity (Maestroni and Conti, 1997). Consistently, a most recent double blind study investigating the myeloprotective effect of melatonin given in combination with carboplatin and etoposide to lung cancer patients shows that melatonin seems to increase the time of chemotherapy induced neutropenia (M. Ghielmini, in preparation). This would confirm the effect of melatonin in tumor-free mice and constitutes important evidence for a potentially dangerous adverse effect of melatonin. These surprising effects reveal the existence of complex neuroendocrine mechanisms regulating hematopoiesis.

Conclusion

The message of this review is that, in analogy with the immune system (Feldman et al., 1987; Malec and Nowak, 1988; Spengler et al., 1990; Felten and Felten, 1991; Geenen et al., 1992; Felten, 1993), neural and neuroendocrine factors should be considered as important hematopoietic regulators. It is probable that beside catecholamines and melatonin many other factors may affect hematopoiesis. For example, it has been recently reported that substance P and neurokinin-A may induce both positive and negative hematopoietic regulators in bone marrow stroma (Rameshwar et al., 1994; Rameshwar and Gascon, 1996). This subtle regulation of the blood forming system might be even more fundamental than that exerted by the cytokine network. These findings indicate, in fact, that the endogenous release of multiple hematopoietic regulators may be controlled by neural or neuroendocrine factors. We might be before the tip of an iceberg representing a mechanism of hematopoietic regulation capable of transducing environmental information to the blood-

forming system. What appears as a new, fascinating research avenue calls for further studies. A central question is whether the neural regulation of hematopoiesis plays any role in aplastic anemia, leukemia, immune-based diseases or during emergencies such as acute infections and/or stress events. Any positive answer to this question might provide a conceptual framework in which new pharmacological strategies to prevent or correct pathological situations could be devised.

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