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REVIEW ARTICLE

Hematopoietic Regulators: Redundancy or Subtlety?

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HEMATOPOIESIS requires a highly complex series of cellular events in which a small population of stem cells needs to generate continuously large populations of maturing cells in eight major lineages. Normally, the diverse proliferative, differentiative, and maturation events required to achieve this occur with precision, which leads to the expectation that the regulatory mechanisms involved would need to be complex. It can also be anticipated that the entry of mature cells into the circulation, their selective localization in appropriate tissues, and their functional activation are also events requiring sophisticated regulatory control.

Given the likely complexity of these events and the knowledge that much of this regulation is achieved by the use of regulatory molecules that can be humoral or cell-associated, it is not surprising that many such regulatory molecules have been characterized, purified, and produced in recombinant form. The known regulators with proliferative effects on one or other hematopoietic population already exceed 20 in number and to these need to be added a variety of inhibitory factors and a number of factors allowing selective cell-cell adhesion. Many additional candidate factors are in the early phase of characterization.

This degree of complexity in biologic processes is familiar enough to those who have addressed the details of coagulation or complement activation. However, for some of those working with the proliferative hematopoietic regulatory factors, there are aspects of the regulators so far characterized that have a raised a growing conviction that these regulators may exhibit a high degree of redundancy. In short, there appear to be more regulators with similar or overlapping actions than would seem to be really necessary to achieve the required cell proliferation.

EVIDENCE FOR REDUNDANCY

There are several types of observation that support the contention that there could be redundancy amongst the proliferative hematopoietic regulators.

Common proliferative actions. Hematopoietic cell proliferation cannot occur spontaneously and needs stimulation by specific regulatory factors.[1] The nature of the events occurring is shown more clearly in semisolid than in liquid cultures because the semisolid matrix allows the progeny of individual precursor cells to remain physically localized during the process of colony formation.

Although most of the proliferative regulators can stimulate one or another type of colony formation in vitro, it needs to be emphasized that the precise pattern of colony formation stimulated by each purified regulator is quite distinctive. No two regulators stimulate exactly the same pattern of colony formation as judged either by colony numbers or, more importantly, by the lineage and maturation pattern of the cells making up the developing colonies. For example, each of the four colony-stimulating factors (CSFs) stimulates the formation of a quite distinct array of colonies.[2-5] However, if these obvious differences are ignored and attention is focused on one particular colony type, then it becomes both intriguing and puzzling to realize that more than one factor can stimulate the formation of what appears to be exactly the same type of colony. For example, the development of small maturing neutrophilic granulocytic colonies can be stimulated, apparently by direct action, by granulocyte-CSF (G-CSF), granulocyte-macrophage–CSF (GM-CSF), macrophage-CSF (M-CSF), Multi-CSF (interleukin-3 [IL-3]), stem cell factor (SCF), and IL-6. Similarly, at least seven factors can stimulate or potentiate the formation of megakaryocyte colonies of one type or another (Fig 1) and a similar situation exists for eosinophils and mast cells.

From the limited viewpoint of achieving in vivo the proliferation of granulocytes or megakaryocytes, the existence of

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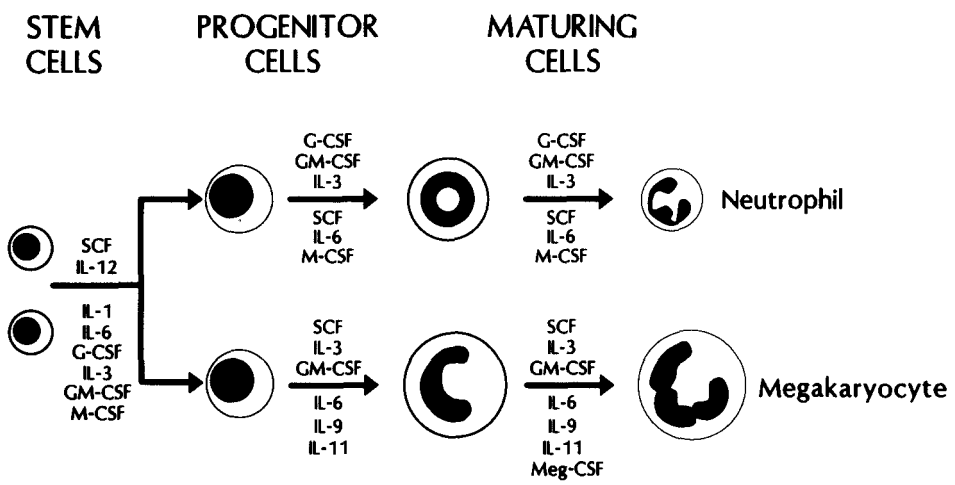

Fig 1. Multiple regulators have actions controlling the formation by stem cells of progenitor cells and, similarly, in the formation of neutrophils or megakaryocytes, six or seven regulators have been shown to have some proliferative effects.

six or seven regulators able to accomplish this process seems unnecessary. The arrangement appears not only to be redundant but to entail an inbuilt risk of dysregulation if differing signals can induce the synthesis of these different proliferative factors.

Common spectra of biologic actions. The action of individual hematopoietic regulators is usually not restricted to cells of a single lineage. Even erythropoietin, possibly the most restricted regulator, with its dominant action on mature erythroid precursors,[6] probably also has some action on megakaryocyte precursors.[7] At the other extreme, Multi-CSF (IL-3) has obvious actions on stem cells, erythroid, granulocytic, macrophage, eosinophil, megakaryocyte, mast, and probably B-lymphocyte precursors.[5,8] It is these cross-lineage actions that lead to the accumulation of multiple regulators with actions on cells in an individual lineage.

What has been more intriguing has been the growing awareness that some regulators have an astonishing range of responding target cells. The regulators concerned are leukemia inhibitory factor (LIF),[9] IL-6,[10] oncostatin-M (OSM),[11] and IL-11.[12] Although the overlap in their range of biologic actions is not complete, nevertheless two or more of these have similar actions in stimulation of platelet formation, induction of differentiation in myeloid leukemic cells, stimulation of hepatocytes to produce acute-phase protein, alteration of neuronal signalling, and suppression of lipid transport to adipocytes. Apart from a general dilemma in understanding the rationale for the existence of regulators with such a bizarre range of target cells,[13] it is equally intriguing that there should be a set of such regulators with a comparable and overlapping range of actions. These similarities extend to in vivo actions because, for example, the injection of LIF, IL-6, or IL-11 induces broadly similar increases in platelet numbers in terms of their speed of development and magnitude.[14-16]

Shared receptor subunits. Recent data derived from the cloning of cDNAs encoding the specific membrane receptors for the hematopoietic and other growth factors have shown the existence of two distinct families of related receptors: (1) tyrosine kinase receptors, including those for epidermal growth factor, M-CSF,[17] and SCF[18]; and (2) hematopoietic receptors, not containing a tyrosine kinase domain, but exhibiting obvious homology in their extracellular domains.[19] This latter group includes the receptors for erythropoietin,[20] GM-CSF,[21] Multi-CSF,[22] G-CSF,[23] IL-4,[24] IL-5,[25] IL-6,[26] IL-7,[27] LIF,[28] and IL-2.[29] The relatedness of receptors in this latter group certainly raises the likelihood that these receptors are derived by evolutionary divergence from a common ancestral receptor.

What has been even more interesting is that most of this latter group of receptors exist in high-affinity form as heterodimers. After ligand binding, the specific α-chains referred to above become associated with at least one other receptor chain (the β-chain). The intriguing aspect of this arrangement is that the β-chains involved are promiscuous. Thus the α-chains for GM-CSF, Multi-CSF, and IL-5 share the same β-chain[30] and, although the exact arrangement remains to be clarified, receptor complexes for IL-6, LIF, OSM, and IL-11 all share a common β-chain, ie, the gp130 molecule.[31-33] Because the β-chain is prominent in initiating intracellular signalling, this arrangement offers an appealing structural explanation for the observations that GM-CSF, IL-3, and IL-5 all have in common the ability to stimulate eosinophil proliferation and that LIF, IL-6, OSM, and IL-11 exhibit shared pleiotropic actions.

However, the converse aspect of this promiscuity is that the arrangement seems to document the existence of true redundancy. If one assumes for the moment that the β-chain is wholly responsible for signalling, then there would seem to be little need for the existence of three distinct α-chains on eosinophils or for three separate regulatory factors to activate such α-chains.

Coexpression of regulator receptors on individual cells. One way of explaining this apparent redundancy would be to propose that major heterogeneity exists within populations of each lineage at each differentiation stage. Perhaps only a subset of granulocytic progenitor cells expresses receptors for G-CSF, another subset of progenitors only receptors for GM-CSF, and so on. Each regulator might therefore act on its own "private" subset of responding cells. An extension of this concept might even propose that distinct subsets of mature granulocytes exist, each with

a distinctive functional capability. Differing emergency circumstances might then require the use of different regulators to stimulate the formation of the most appropriate subset of mature granulocytes to respond to the special requirements of a particular situation.

Present information is not quite complete enough to absolutely exclude this heterogeneity hypothesis. Nevertheless, in the case of the CSFs, a variety of data from autoradiographic studies using radiolabeled regulators[34-36] and the reciprocal transfer of developing colonies from one stimulus to another[3,37] suggest that the more likely situation is that a majority of cells, whether progenitor cells or maturing progeny, coexpress receptors for more than one regulator.

Indeed, the ability to document interactions between different regulators (either downregulation[38] or upregulation[39]) and a variety of competitive or enhancing interactions between pairs of regulators necessitates the coexpression of more than one type of receptor on individual cells. Any interpretation of the significance of multiple regulators with similar actions therefore needs to encompass the fact that multiple regulators can and do act on individual cells.

INTERPRETATIONS OF THE SIGNIFICANCE OF APPARENT REDUNDANCY

Consideration of these observations has led many to conclude that the hematopoietic regulators do indeed exhibit significant redundancy. Predictably, this possibility has elicited a variety of responses. Some have mentally shrugged and concluded that, given our evolutionary origin, it is inevitable that "genetic debris" will persist in the most advanced organisms. This view would hold that some of today's "regulators" in fact perform no real function in the body, even though the regulators encoded can be shown still to have actions on present day cells. This conclusion seems illogical. If such regulators can act, then there are presumably situations in which they do act.

A less extreme response is to propose that a hierarchy of regulators exists, some with a mandatory role in daily cell production and others with weaker actions or perhaps only becoming important under emergency circumstances. A rather naive corollary of this view, implicit in many publications, is that if a factor is usually present in the circulation, it is more likely to be a major regulator than one less often or rarely detectable in the circulation.

A more appealing concept is that multiple regulators exist because they are designed to act sequentially within a particular lineage, some preferentially acting early in a differentiation sequence, others acting later in the sequence. Unfortunately this concept is not well supported by available data. Erythropoietin is perhaps the best candidate for such a sequentially acting regulator, having its most obvious action on the most mature erythroid precursors,[6] whereas IL-3[5] and SCF[40] clearly are able to act earlier in the erythroid lineage. Similarly, IL-5 may act primarily on more mature eosinophil precursors[41] and IL-6 may act particularly on more mature megakaryocyte precursors.[42] However, these likely examples of sequential action are few in number and the bulk of the hematopoietic regulators exhibit little evidence of stage restriction. For example, each of the four CSFs has actions on cells ranging from the stem cell to fully mature cells.[43] The notion of sequential action has done little therefore to resolve the redundancy problem.

EFFECTS OF DELETION OR SUPPRESSION OF HEMATOPOIETIC REGULATORS

One might suppose that the most definitive approach to establishing whether a particular regulator is genuinely redundant would be to suppress the regulator either using specific antibodies or by generating animals in which the gene in question has been deleted or functionally inactivated. If some abnormality develops in hematopoiesis as a result of either procedure, clearly the factor concerned is not redundant and its loss cannot be compensated for by the overlapping action of another factor.

In early studies, injection of antibodies to erythropoietin suppressed erythropoiesis,[44] indicating that the action of erythropoietin cannot be redundant. Somewhat comparable data have been generated by Hammond et al[45] from an analysis of dogs developing antibodies to injected human G-CSF that cross-neutralize canine G-CSF. Such dogs developed neutropenia, indicating that G-CSF plays an important role at least in the basal production of granulocytes, a role that is not able to be compensated for by other granulocyte-active factors such as GM-CSF or Multi-CSF.

In parallel studies, mice with a genetic defect resulting in osteopetrosis (op/op) have been recognized to have an inactivating nucleotide insertion in the M-CSF gene preventing the production of M-CSF.[46] Such mice exhibit a major deficiency in macrophage-derived osteoclasts and partial deficiencies in other macrophage populations, abnormalities correctable by the injection of M-CSF.[47] Again, the conclusion is able to be drawn that M-CSF is essential for the formation of osteoclasts and some macrophages. Because op/op mice are not completely devoid of macrophages, the formation of at least some macrophage populations must be able to be stimulated by other factors, such as GM-CSF.

At present, a number of groups are developing mice with deletion or inactivation of other hematopoietic growth factor genes such as GM-CSF or IL-3 and data from such animals may provide a much clearer view of the redundancy question.

However, some caution needs to be exercised in interpreting such data. Studies with LIF-deleted mice illustrate this comment. As mentioned above, the curious pleiotropic effects of LIF are duplicated to varying degrees by the actions of three other regulators, IL-6, OSM, and IL-11, leading to an expectation that deletion of the LIF gene might result in no observable effects. Superficially, this has indeed been what was observed, in the sense that living LIF-deleted animals can be generated.[48,49] However, such mice have been reported to be somewhat undersized and to have reduced numbers of stem and progenitor cells in the spleen and to a lesser degree in the marrow,[49] raising the possibility that some hematopoietic cells do depend for optimal performance on a special action of LIF. More importantly, LIF-deleted mice are unable to become pregnant.[48,49] In normal pregnancy, cells in the uterine wall adjacent to the implan-

tation site exhibit a marked increase in the transcription and production of LIF at the time of implantation.[50] This appears to be essential for implantation because LIF-deleted blastocysts can implant normally in LIF-producing mice and the implantation defect in LIF-deleted mice can be corrected by the injection of LIF. So, is LIF a redundant or a nonredundant regulator? The answer depends entirely on the context. Some functions of LIF may be redundant whereas at least one must be unique and crucial for pregnancy to occur.

The ability of the deletion or inactivation approach to obtain answers to the redundancy problem may in fact be a more difficult task than was envisaged by the exponents of this approach. Deletion of a growth factor gene may well induce no obvious abnormality until a particular challenge situation is applied, when a deficiency then becomes evident. Worse, it may become necessary to perform the observations at a quite restricted time after deletion. It has recently been reported that the dramatic defects in op/op mice resolve spontaneously as the animals age.[51] This suggests that, although compensating mechanisms may require some time to become operative, genuine defects may be missed if analysis is delayed too long.

My own expectation is that the present series of gene inactivation experiments may generate some initially misleading data but will ultimately result in the conclusion that most of the factors under discussion have both unique and redundant actions, depending on the circumstances analyzed.

REGULATOR COMBINATIONS ARE NECESSARY FOR OR ENHANCE HEMATOPOIETIC CELL PRODUCTION

A more positive approach to the redundancy question is to carefully analyze the consequences of using combinations of regulators compared with the effects of individual regulators, when acting alone. Are there cell populations requiring costimulation by different regulators for proliferation? Do regulator combinations induce enhanced proliferative responses? On this latter question, if equivalent concentrations of two mutually redundant factors were combined to stimulate a target progenitor cell population, one of two outcomes should be observed. The combination might exhibit the same proliferative stimulation as either acting alone or, more likely, the combination would stimulate proliferation equivalent to that stimulated by twice the concentration of either factor when acting alone.

Proliferative responses can most readily be analyzed in simplified culture systems and here the use of clonal cultures is particularly revealing because two quite different parameters can be distinguished: alteration in colony size and alteration in colony numbers. As shown in Fig 2. enhanced cell production can be based on two quite different processes and it is suggested that separate terms be adopted to distinguish between the two. Increased colony size can be labeled "synergy." This is the process by which two or more active factors, acting on the same progenitor cell, induce the formation of larger number of progeny. The second process could be termed "recruitment." In this process, the combined actions of two or more regulators allow increased numbers of progenitor cells to proliferate either because distinct subsets of progenitor cells exist that respond exclusively to one factor or because some progenitors require simultaneous stimulation by two or more factors before being able to respond.

Stem cells purified to homogeneity by fluorescence-activated cell sorter (FACS) fractionation characteristically are not able to proliferate if stimulated by single regulatory factors. Combinations of two or more regulators including SCF or IL-12 with IL-3, IL-6, and G-CSF are required before proliferation occurs.[52-54] This appears to be a clear example of recruitment (the need for simultaneous signalling by two or more regulators), but the actual cellular processes could be quite complex. Information remains incomplete on what regulator receptors are normally expressed on murine stem cells. Certainly receptors for SCF are present and, in studies in progress in this laboratory, receptors for IL-1, IL-3, IL-6, and G-CSF appear to be present in somewhat low numbers on at least a proportion of purified stem cells (C.-L. Li, G.R. Johnson, W. McKinstry, unpublished data, May 1993). SCF, when acting alone, maintains the survival of the stem cells but induces little cell division[53] and SCF may act to induce or increase expression of other receptors whose activation by the appropriate regulator is then necessary for cell division.

In contrast, the enhancement interactions noted with unfractionated or FACS-purified granulocyte-macrophage progenitor cells more often appear to represent synergy. For example, when two CSFs are combined, there is not an additive increase in resulting colony numbers, presumably because most progenitors are able to respond to stimulation by either factor when acting alone. However, what does occur is an increase in cell numbers in the colonies that is somewhat greater than achievable by using twice the concentration of either factor alone.[4,55,56]

Several mechanisms could explain synergy. Activation of additional receptors on a cell by the use of two factors is likely to enhance the mitotic signal because of coalescence of initially differing signalling pathways into a common final pathway reaching the nucleus.[57] Superadditive responses to regulator combinations could occur if signalling from one type of receptor is being restricted by limiting concentrations of some signalling intermediate. This deficiency might be overcome by supplementation of signalling intermediates from a second type of activated pathway. It has also been documented that activation of one set of receptors can enhance expression of receptors for other regulators, allowing enhanced responsiveness to the second regulator.[39] The occurrence of superadditive synergy is not the response expected from the combination of two redundant factors and is thus an argument against such redundancy.

A more dramatic enhancement of cell proliferation is observed when SCF is combined with G-CSF, GM-CSF, Multi-CSF, or IL-6 to stimulate cultures.[58-60] Again, there is not an additive increase in total colony numbers but there is a consistent 10- to 20-fold increase in mean numbers of cells per colony. Analysis of this phenomenon has shown that the key process relates to the behavior of blast colonies in the

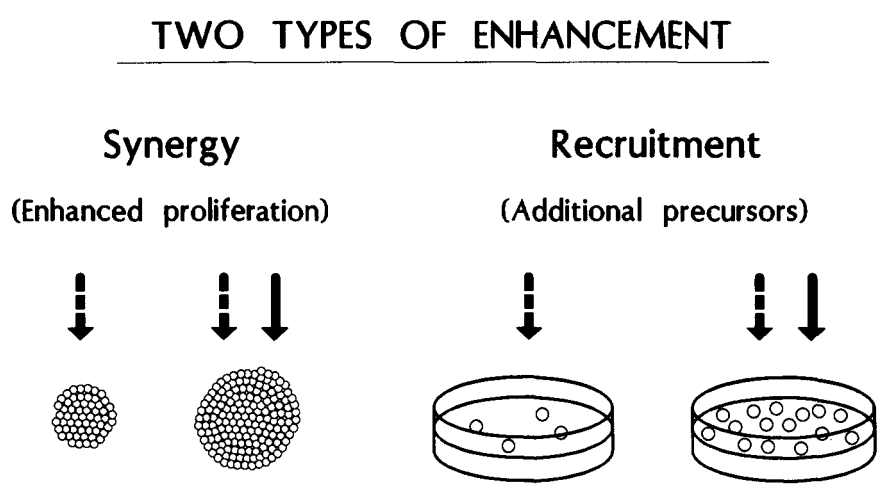

Fig 2. Combination of two growth factors can stimulate the formation of increased numbers of progeny by two distinct mechanisms. In synergy, the combined action of two factors, each of which is active alone, amplifies cell production by individual clonogenic cells. In recruitment, increased cell production is achieved by activating cell production by additional clonogenic cells either because each regulator stimulates differing subsets of clonogenic cells or because some clonogenic cells require double signalling before being able to proliferate.

cultures. These are formed (albeit rather ineffectively) by relatively mature stem cells in response to stimulation by SCF, possibly with the assistance of low levels of endogenously produced regulators of other types.[59] Deliberate addition of a CSF or IL-6 to SCF-stimulated cultures does not increase the number of these blast colonies but greatly amplifies the number of cells present in them.[60,61] The cells in blast colonies are in fact committed progenitor cells for various hematopoietic lineages, so, in this situation, enhancement is occurring by the process of synergy. However, the resulting progenitor cells are quite heterogeneous and many are in lineages not able to be stimulated to proliferate by the SCF-CSF combination used to initiate their formation.[60,61] Thus, for example, the combined action of SCF plus G-CSF leads to the production of large numbers of macrophage-committed progenitor cells. To reap the benefit of these additional progenitor cells in terms of mature cell production, the use of an additional macrophage-active factor becomes mandatory. Thus, a process that involves synergy results in a situation requiring recruitment to yield the maximum numbers of maturing progeny.

The data discussed indicate that, for optimal progenitor cell production by stem cells, at least two factors are needed and then, for optimal production of maturing progeny from these progenitor cells again, more than two factors are needed. These phenomena probably document some of the reasons why multiple regulatory factors exist. That this phenomenon is not a curious in vitro artefact has become evident from studies in vivo. The injection of G-CSF induces impressive increases in blood granulocyte levels but, curiously, also induces up to 100-fold increases in peripheral blood levels of progenitor cells in all lineages.[62] Use of G-CSF in mice with the Steel or W^v mutations that lack the ability either to produce SCF or SCF receptors is much less effective in stimulating granulopoiesis and in elevating progenitor cells,[63] allowing the conclusion that G-CSF responses are in fact SCF–G-CSF responses. The puzzling increase in "inappropriate" progenitor cells in response to G-CSF is then an example of the consequences of the combined action of SCF and G-CSF on stem cells as analyzed in vitro. The implication is that the clinical use of additional, more broadly acting regulators in such patients might achieve significant increases in monocytes, eosinophils, and erythroid cells, based on the increased numbers of progenitor cells now available for stimulation.

Not all combinations of factors lead to enhanced responses. Thus, combination of SCF with M-CSF does not result in striking enhancements.[60] Furthermore, at least one combination, that of GM-CSF with M-CSF when acting on murine cells, actually results in a significant decrease in the formation of certain macrophages compared with that able to be elicited by M-CSF acting alone.[4,64] This is not likely to be the only example of a suppressive consequence after regulator combination and this potential outcome needs to be kept in mind.

Most data indicate that stem cell proliferation requires costimulation by multiple regulatory factors, whereas progenitor cells can be stimulated effectively (if not optimally) by single regulators. However, it needs to be commented that there are some progenitor cells whose proliferation requires costimulation. For example, there is a subset of granulocyte-macrophage progenitor cells able to produce large numbers of progeny, but such proliferation requires the use of two or more CSFs.[4] Similarly, the development of maximal numbers of megakaryocyte colonies requires the combination of Multi-CSF (IL-3) with IL-6 and SCF. Thus, there appear to be situations in which it is not merely more efficient to use receptor combinations to stimulate cell formation but the use of combinations is actually mandatory. There is no reason to suppose that the same principles do not apply for the production of basal numbers of mature cells. It may always be more efficient to use multiple factor combinations to achieve any level of cell production.

A quite distinct possible advantage from the use of regula-

tor combinations may be the lower demands placed on factor-producing cells and their induction systems. It is fairly common for individual cells such as lymphocytes,[65] endothelia,[66] or stromal cells[67] to simultaneously produce more than one type of regulatory molecule in response to a single type of inductive signal. It has yet to be established whether there is a significant limitation in the ability of a cell to produce any one factor. If so, an arrangement lowering the required concentrations of each factor by using them in combination might usefully reduce demands on factor-producing tissues.

At the clinical level, the increased cell production able to be stimulated by combinations of factors compared with that achievable using single factors would have some potential advantages. Less of any one factor would need to be used, probably reducing the likelihood of adverse responses and possibly reducing costs of therapy.

REGULATOR COMBINATIONS ACHIEVE BROADER CELLULAR RESPONSES

It is now well recognized that all regulators are able to influence hematopoietic cells of more than one lineage, but the breadth of such actions differs widely. For example, at least when acting on committed progenitor cells, G-CSF action is largely restricted to granulocytic populations and M-CSF action is similarly restricted to monocyte-macrophage populations. In contrast, both GM-CSF and Multi-CSF can stimulate the production of a much broader range of hematopoietic cells. We remain quite ignorant regarding the range of cell types required for optimal responses to various emergencies such as tissue injury or infections, but for most common emergencies, it can be presumed that an optimal response does require the involvement of cells from more than one lineage. Combination of two or more CSFs in vitro increases the range of responding cells[4] and such combinations could therefore be expected to elicit broader, and thus more useful, responses in at least some clinical situations.

When various CSFs were injected into animals, the range of responding cells faithfully reproduced the range of actions established by earlier in vitro studies. However, a further subtlety emerged from these studies best exemplified by comparing the responses to G-CSF and GM-CSF when injected into the peritoneal cavity. G-CSF elicited little increase in peritoneal cell numbers but major increases in peripheral blood granulocytes.[43] In contrast, with the doses used, GM-CSF induced only minor increases in blood cell levels but a remarkable increase in peritoneal cell macrophages and substantial increases in peritoneal eosinophils and neutrophils.[68] Combination of both factors retained the distinctive responses of the individual factors with significant enhancement of cell numbers in both locations.

This model system suggests that the organ distribution of cells stimulated to develop by different regulators differs and that combinations of regulators should achieve a wider tissue distribution of responding cells. It is also likely, in certain situations such as a local infection, that high levels of one CSF, such as GM-CSF, may be produced locally while systemic levels of G-CSF become elevated. In combination, this would represent a quite sophisticated system for focusing the additional cells being produced to the location where they are most required.

REGULATOR COMBINATIONS AND DIFFERENTIATION COMMITMENT

In addition to stimulation of cell production, regulators can also induce differentiation commitment in responding cells.

Some of the clearest examples of the advantages of regulator combinations concern the induction of differentiation commitment and/or maturation in leukemic cell lines. Thus, some level of differentiation commitment can be achieved in HL60 or U937 leukemic cells by GM-CSF, G-CSF, IL-6, or LIF when acting alone. However, combinations of two of more of these elicit enhanced suppression of clonogenic cells and this principle is observable with other leukemic cell lines.[69,70] Unlike the situation with mitotic signalling, it is not yet apparent whether different differentiating-inducing factors necessarily initiate a common signalling pathway. Indeed, in a recent study of the action of LIF, IL-6, and OSM on M1 myeloid leukemic cells, overexpression of the helix-loop-helix gene SCL resulted in clear differences in the subsequent response of these cells to signalling by these three regulators even though the receptors concerned share a common gp130 subunit.[71]

Again, there are situations when regulator combinations can be antagonistic. Thus, GM-CSF and M-CSF can compete in vitro for commitment of bipotential progenitor cells to the granulocyte or macrophage lineage.[37,72] However, it is likely that, in vivo, the reserve of stem and progenitor cells is sufficient to compensate for such competitive actions.

COMMENTS

For the various reasons discussed above, it seems incorrect to regard the multiplicity of hematopoietic regulators as representing a highly redundant control system of dubious value. There is increasing evidence of the necessity to use regulator combinations to achieve certain types of cell production and, for others, of the higher efficiency of multiple regulatory factors, even when their actions appear to exhibit considerable overlap. These advantages also include the ability to achieve more subtle localization of cells produced and to achieve the complex mixtures of the cells required in certain situations (Fig 3).

If the existence of multiple regulatory factors indicates subtlety rather than redundancy, more effort needs to be expended to determine exactly what is achievable by the use of regulator combinations. This requires in vitro experiments that are demanding to design and difficult to publish in digestible form. However, the situation for our clinical colleagues is much worse. Some 20 regulators are available in recombinant form for clinical testing and the resources of suitable research units and patients will be strained to accomplish the required trials on individual agents. If the reality is that regulators are more efficient and effective when used in combination, the situation becomes a logistical nightmare. Even using a single dosage schedule, there are

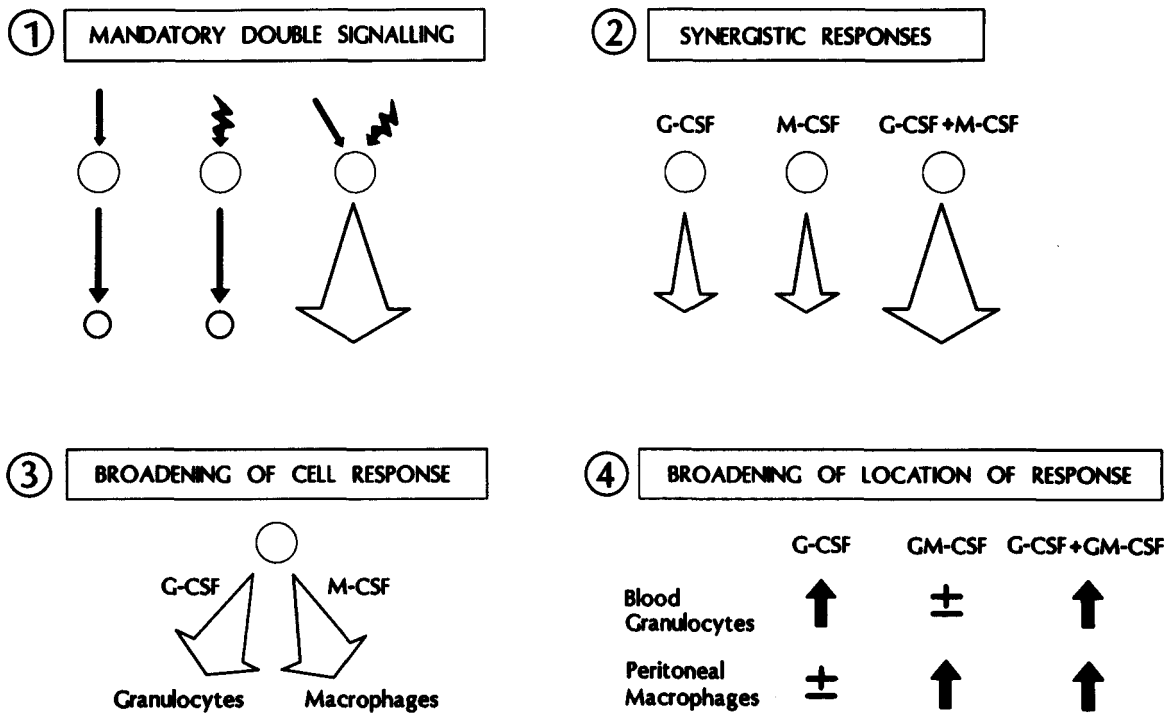

Fig 3. Four examples of the advantages of using multiple regulators. (1) Stem cells and some progenitor cells require double signalling for proliferation. (2) Combinations of factors can elicit superadditive synergistic proliferative responses. (3) Combination of two factors broadens the lineage range of maturing cells produced. (4) Combinations of factors can broaden the location of cells responding or accumulating after increased cell production.

more than 1 million possible combinations of 20 agents and testing of random combinations is impossible.

It may be possible to predict from in vitro data whether certain combinations might be of clinical value. Unfortunately, existing laboratory data are quite inadequate to cover all possibly valuable combinations and there is an urgent need to extend these laboratory data lest some quite unexpected favorable combinations are overlooked. A better understanding of the basic cellular principles involved in responses to regulators may help us in this dilemma.

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