Regulation of Hemopoietic Cell Differentiation and Proliferation

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Differentiation and proliferation of almost all hemopoietic cell lines can now be studied in vitro. Cloning techniques and suspension cultures allow the study of proliferation of the multipotential hemopoietic progenitor cell and the committed progenitors for granulocytes, macrophages, eosinophils, megakaryocytes, and erythrocytes. The proliferation of each of the committed progenitor cells is controlled by specific glycoproteins and two of these have recently been purified: granulocyte-macrophage colony-stimulating factor (GM-CSF) and erythropoietin. The rate of proliferation of the GM-progenitor cells and their pattern of differentiation depends on the concentration of the hormone. At low concentrations of GM-CSF (10^{-11} M) fewer progenitor cells are stimulated and macrophage colonies rather than granulocyte colonies develop. The change in the direction of granulocyte-macrophage differentiation appears to be related to a) the concentration of GM- CSF and b) the different sensitivity of a subpopulation of monocyte colony-forming cells which are responsive to GM-CSF even at low concentrations of the regulator. Analysis of the rate of RNA synthesis by bone marrow cells has shown that GM-CSF stimulates the mature nondividing end cells of differentiation (ie, polymorphs) as well as the progenitor cells. Although GM-CSF and erythropoietin have been radiolabeled, binding studies have been hampered by the loss of biologic activity during the labeling procedure and the heterogeneity of the target cells to which the regulators bind. Surface proteins and receptors for erythrocytes have been well characterized but the relationships between these proteins and the cell surface proteins of nucleated blood cells is not well understood. It appears that some proteins are lost from the cell surface during the development of granulocytes, which are retained on the surface of the B lymphocyte. Other proteins such as chemotactic receptors and complement receptors only appear on the mature cells. External radiolabeling of the granulocyte surface using iodogen yielded a simple profile of ^{125}I-labeled proteins when analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Key words: hemopoiesis regulation, hemopoietic cell differentiation, erythropoietin, erythropoiesis, cell surface labeling, polymorphonuclear leukocyte, granulocyte-macrophage colony-stimulating factor

GENERAL ASPECTS OF HEMOPOIETIC DIFFERENTIATION

In common with all eukaryotic differentiation, hemopoiesis involves the development of specific cell functions from a multipotential cell [1]. A general scheme of hemopoietic differentiation is outlined in Figure 1. The multipotential cell is not only self-replicating, but it produces a set of immature progenitor cells committed to a specific line of differentiation. Each of the committed cells proliferates and differentiates along a defined pathway, giving rise to a large number of functional hemopoietic cells. The multipotential hemopoietic cell can be activated from a quiescent state into DNA synthesis [2] whence it can divide, reproducing itself, or give rise to progeny that are committed to a specific hemopoietic cell line [3, 4]. It is not yet clear whether there are cells with limited commitment (ie, to two or more cell lines), which are the progeny of the multipotential hemopoietic cell and the precursors of the progenitor cells for a specific cell line [5]. Most committed progenitor cells appear to be in a cell cycle of relatively short duration [6], but their proliferation in vitro is completely dependent on adequate concentrations of specific regulatory proteins, eg, for granulopoietic progenitors the glycoprotein colony-stimulating factor [7–9] and for erythroid progenitors, erythropoietin [10]. Some reports indicate that multipotential cells can also proliferate in vitro in the presence of monolayers derived from embryonic fibroblasts [11] or adult bone marrow [12]. However, no evidence has yet been reported which indicates that multipotential hemopoietic cell determination can be influenced by protein regulators. Individual fetal multipotential cells have been recently observed to produce a range of committed progenitor cells [13].
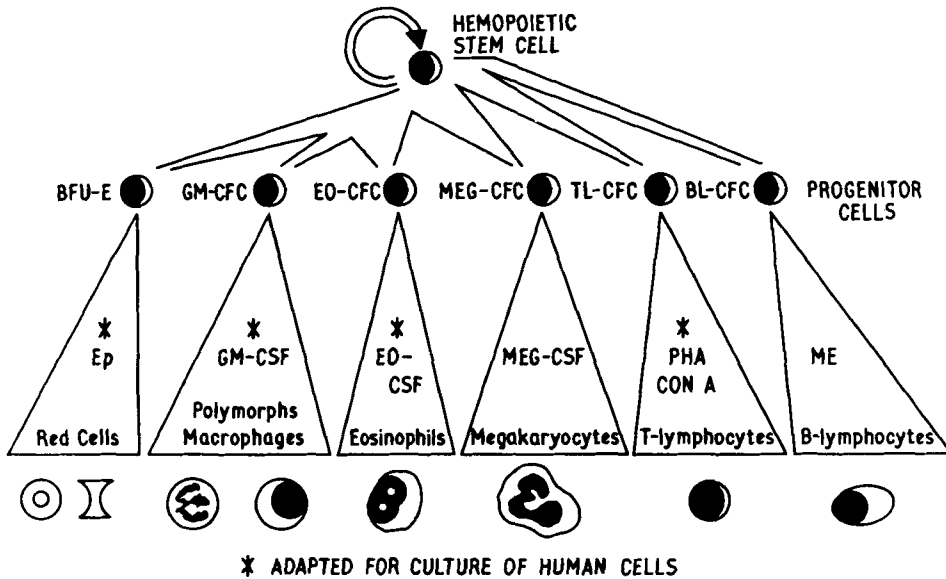

Fig 1. Schematic representation of hemopoietic differentiation. The multipotential hemopoietic stem cell produces progenitor cells committed to a specific line of differentiation: Burst-forming unit-erythroid (BFU-E-), granulocyte-macrophage (GM), eosinophil (EO), megakaryocyte (MEG), T lymphocyte (TL), B lymphocyte (BL) colony-forming cells (CFC's). The differentiation of the progenitor is stimulated by erythropoietin (Ep), GM, EO, and MEG colony-stimulating factors. Lymphocyte progenitors are stimulated by phytohemagglutinin (PHA), concanavalin A (Con A), and 2-mercaptoethanol (ME).

A conditioned medium containing the glycoprotein regulators necessary for the expression of the differentiation program for the megakaryocyte, eosinophil, granulocyte, macrophage, and erythroid progenitor cells produced clones containing all of the corresponding cell types from a single fetally derived hemopoietic cell [13].

The expression of the committed differentiation programs for erythroid (E) progenitors and granulocyte macrophage (GM) progenitors requires the presence of erythropoietin [14–16] or granulocyte-macrophage colony-stimulating factor [17, 18], respectively. The kinetics of proliferation and patterns of cellular differentiation of cells derived from the E or GM progenitor cells are dependent on the concentration of their appropriate regulators. Very few properties of most of the differentiated hemopoietic cells have been investigated in detail. However this deficiency is, in part, alleviated by the intensive study of erythropoietic differentiation, eg, the terminal stages such as the control of hemoglobin synthesis [19, 20] and earlier stages using mouse erythroleukemic cells induced by Friend leukemia virus [21, 22]. Several mutant lines of these cells have been derived that have been used to define the terminal stages of erythroid differentiation at a molecular level [23]. Knowledge of the surface properties of lymphoid cells [24, 25] far exceeds our information of the surface properties of the other leukocytes. However, some functional surface receptors have been defined for polymorphonuclear granulocytes [26], eosinophils [27] and macrophages [28], but as yet there is no information concerning the structure or mode of action of these receptors.

Hemopoiesis can be manipulated in vitro by taking advantage of selective culture systems and by controlling the concentration of the differentiation factors. The culture systems presently used for the study of hemopoiesis, the state of our knowledge of the proteins regulating the differentiation of each cell line, the effect of the regulators on the metabolism of their target cells, and the expression of surface antigens on progenitor and differentiated hemopoietic cells will form the basis of this review. It will become obvious that there are still no answers to the questions concerning the determination of cells to a specific pathway of differentiation, and even though there is considerable phenomenological information about the cellular and molecular events expressed during differentiation, we still know little of the mechanisms by which regulators act. There are, however, the culture techniques and some of the regulators available to probe these questions.

HEMOPOIETIC CULTURE SYSTEMS

Highly purified populations of some hemopoietic cells may be harvested directly from animals, but the hemopoietic cells available are a mixture of both mature and immature cells from various lines of differentiation. Many of the cells can only be identified by functional tests in vitro and in vivo, and many of the culture systems have been used mainly for analytic purposes.

Suspension Cultures

Several suspension culture systems have been developed; for example, the Friend virus-induced erythroleukemic cells have made it possible to harvest large numbers of cells at different stages of differentiation and to study their biochemical status. This is not easily achieved for many normally differentiating cells, but large-scale granulocyte and macrophage production can now be simulated in suspension cultures using bone marrow progenitor cells and granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF appears to stimulate mouse bone marrow GM-progenitor cells to divide, giving

rise to daughter cells which are still responsive to GM-CSF. In the presence of GM-CSF these cells continue to differentiate, giving rise to mature granulocytes and macrophages [29]. Little is known about the surface proteins of granulocytes, macrophages, or their progenitor cells. A recent report indicates that there are proteins shared by human B lymphocytes and the GM-progenitor cell [30], myeloblasts, and myelocytes [31] but not by metamyelocytes or mature granulocytes [31]. The liquid culture system should be ideal for determining the fate of proteins present on immature cells and the appearance of specific differentiation markers such as the chemotactic receptors. Another study made use of long-term bone marrow cultures to study the appearance of the immunoglobulin Fc receptor, the C3d complement receptor, and the C3b complement receptor on cells in the granulocytic series [32]. It appears that the Fc receptor is expressed on immature granulocytic cells (myelocytes), while the C3b receptor is only observed on the mature cells [32].

Although the multipotential hemopoietic progenitor cells appear to be lost from most suspension culture systems within two or three days [33, 34], it is possible to maintain hemopoiesis in long-term cultures using bone marrow monolayers [12]. The disappearance of the multipotential cells in liquid culture did not appear to be responsible for the increase in the level of GM-progenitor cells in bone marrow suspension cultures stimulated by GM-CSF [33]. In long-term bone marrow cultures [12] the GM-CSF concentration is so low that it is not easily detectable. When GM-CSF is added to the cultures, there is rapid differentiation of GM-progenitors, an increase in mature granulocytes, and the disappearance of multipotential cells [35]. The multipotential stem cells may disappear because their rate of determination (to replace the progenitor cell) exceeds their rate of self-renewal. This indicates that there may be a feedback mechanism which operates between the committed progenitors and the multipotential hemopoietic cell. It may be this feedback which controls the rate of determination of the multipotential hemopoietic cell along a given cell line. No attempts have been made so far to detect factors present in the long-term cultures which might suppress the rate of multipotential hemopoietic cell determination.

The terminal pattern of GM-progenitor proliferation and differentiation in vitro does not appear to be influenced by high concentrations of erythropoietin [35a], nor does erythroid differentiation appear to be altered by GM-CSF [10]. Van Zant and Goldwasser have attempted to show competition between GM-CSF and erythropoietin. Their experiments were performed at very high cell densities with impure preparations of the regulators. Since the reported competition was not quantitatively related to the ratio of the erythropoietin and GM-CSF concentrations, a straightforward interpretation is difficult [35b]. Similarly, other hemopoietic progenitor cells appear to proliferate only in response to their specific CSF (eg, megakaryocyte or eosinophil): a response not effected by GM-CSF [13, 36]. The differentiation of GM-progenitors is altered by the concentration of GM-CSF and by exposure to a macrophage-specific CSF (M-CSF) [37]. At low concentrations of GM-CSF from mouse lung-conditioned medium, colonies contain macrophages only. At higher concentrations more than 85% of the colonies contain granulocytes [38, 39]. When the GM-progenitor cells are initially activated by GM-CSF but subsequently stimulated by the M-CSF only, macrophage colonies develop. Indeed, almost 55% of the GM-progenitors appear to have become committed to granulocyte differentiation after 24-h exposure to GM-CSF and these cells die rather than proliferate in the presence of M-CSF [40]. Interestingly, in the presence of high concentrations of M-CSF (where only macrophage colonies appear to develop) the addition of GM-CSF, at a low concentration

(which in itself would only stimulate macrophage colonies), leads to the production of large numbers of granulocyte colonies [40]. Competition experiments of this type appear to offer a unique experimental opportunity for the study of the molecular aspects of differentiation at the level of determination. It should be possible to analyze the surface changes of some of the GM-progenitor cells in response to different concentrations of M-CSF and GM-CSF. A recent report describing the production of a specific granulocyte-CSF [41] should allow a further definition of the molecular regulation of macrophage and granulocyte development.

Semisolid Clonal Cultures

Culture systems are available for most of the hemopoietic progenitor cells that allow single cells to [42] generate colonies of up to 10,000 cells in 7 days (Table I). Most of these culture systems are regulated by specific glycoproteins; however, the molecules responsible for the formation of B-lymphoid colonies in agar have not yet been characterized. Mouse B-lymphocyte colony formation appears to be potentiated by products released by both sheep red blood cells [43] and mouse macrophages [44], but no attempts have been made to characterize these factors. Similarly, the growth of human T-lymphocyte colonies from peripheral blood leukocytes is stimulated by macrophage monolayers or medium conditioned by these monolayers. There appears to be a protein of 12,000 daltons present in the macrophage-conditioned medium which is responsible for the growth of the T colonies [45]. Johnson and Metcalf recently reported the growth of clones from mouse fetal liver cells that contained erythroid cells, granulocytes, macrophages, megakaryocytes, and eosinophils [13]. These clones were derived from single cells in the presence of medium conditioned by spleen cells which had been stimulated by pokeweed mitogen [46]. This provides evidence for the first time that multipotential hemopoietic cells can undergo the transition to committed progenitor cells in vitro and that the committed cells can

TABLE I. Mouse Hemopoietic Progenitor Cells: Culture, Receptors, and Stimulators

Type of progenitor cell	Culture technique	Surface proteins	Stimulator
Multipotential	Two-phase suspension [11, 12, 34]	Viral antigens [76]	Isoproterenol [2]
	Semisolid clones [13]	Brain antigens [72]	
Committed:			
Granulocyte-macrophage	Two-phase suspension [12] Suspension [33] Semisolid clones [7, 8]	B lymphocyte antigens [30]	GM-CSF [39, 53] M-CSF [40] G-CSF [41]
Erythroid	Suspension [64] Semisolid clones [14]		Erythropoietin [51]
B Lymphocyte	Semi solid clones [43]	Immunoglobulin-Fc Receptor [44]	Mercaptoethanol [43]
Megakaryocyte	Two-phase suspension [35] Semisolid clones [36]	—	MEG-CSF [36]
Eosinophil	Suspension [99] Semisolid clones [46]	—	EO-CSF [46]

then express this differentiation program. It is known that the pokeweed mitogen-stimulated, spleen cell-conditioned medium contains glycoproteins that stimulate the committed granulocyte-macrophage, eosinophil, erythroid, and megakaryocyte precursors [46, 47], but it is not yet known whether there are also proteins able to stimulate the multipotential cell to generate the committed progenitors.

HEMOPOIETIC REGULATORS

Hemopoiesis in vivo is complicated by the many different cell populations and subtle physiological stresses that greatly alter both cell production and tissue distribution of the hemopoietic progenitor cells. As a consequence, much of our knowledge of in vivo hemopoietic regulation is actually inference from experiments conducted in vitro. Erythropoietin is known to function in vivo [48]. GM-CSF has also been detected in vivo [49] and its concentration appears to correlate with its expected function [50], but definitive experiments to prove its activity in vivo have not been reported.

Hemopoietic regulation is controlled both by glycoprotein-stimulating factors, which accelerate both the proliferation of specific precursors, and by differential kinetic responses of subpopulations of the hemopoietic progenitors to these stimulating factors. Four such glycoproteins have now been purified: human urinary erythropoietin [51], sheep plasma erythropoietin [52], mouse lung-conditioned medium GM-CSF [39], and mouse L-cell-conditioned medium GM-CSF [53]. These molecules all occur naturally in low concentrations and require a considerable amount of source material for their successful purification: For example, 2,500 liters of human urine from patients with aplastic anemia was required for the preparation of 10 mg of erythropoietin; 50 liters of anemic sheep plasma produced 100 μg of pure erythropoietin, and 4 liters of mouse lung-conditioned medium or 40 liters of mouse L-cell-conditioned medium gave rise to 100 μg of pure GM-CSF. All of these proteins appear to contain sialic acid [51, 53, 54], but only the GM-CSF's bind to concanavalin A−Sepharose [10, 39, 55]. Human urinary erythropoietin appears to be more active than sheep plasma erythropoietin when assayed in vivo using rats. The apparent molecular weight of native erythropoietin under dissociating conditions was 39,000 [51], slightly lower than that for sheep erythropoietin (46,000) [56]. Some microheterogeneity of the mouse lung GM-CSF appears to be associated with carbohydrate moieties such as sialic acid, glucose and mannose; however, the purified biologically active molecule consists of a single molecular weight species (23,000) [39, 57]. Mouse L-cell-conditioned medium contains several CSF's with different molecular weights and carbohydrate composition as defined by binding to concanavalin A−Sepharose [55]. The GM-CSF species purified from mouse L-cell CSF appears to be a glycoprotein with a molecular weight of 70,000, which can be dissociated into inactive subunits in the presence of thiol-reducing reagents. When attempts are made to radioiodinate erythropoietin, all biological activity is lost [51], even in the presence of dimethyl sulphoxide [51]. There is also a loss of biological activity when either GM-CSF from mouse lung-conditioned medium or GM-CSF from mouse L-cell-conditioned medium are radioiodinated [57, 58]; however, most of the biological activity can be preserved by protecting the molecule with dimethyl sulphoxide during the iodination [59]. Purified GM-CSF appears to be active in vitro at concentrations as low as $10^{-11} - 10^{-12}$ M [51, 39, 53], suggesting that the molecule binds to the cell surface at low concentrations. It should be possible to analyze the binding of ^{125}I-GM-CSF to its target cells in bone marrow using autoradiography. Although the colony-stimulating factors for other cell types have been partially characterized [40, 47], their purifications

have not proceeded to the stage where radiolabeling can be contemplated. It should be possible, however, to use these reagents in competitive binding studies with labeled GM-CSF or erythropoietin to determine whether there is any cross-reactivity at the level of the membrane receptors for the hemopoietic differentiation factors. It will be particularly useful to have CSF's specific for a given hemopoietic cell type. Macrophage-CSF is available from yolk sac-conditioned medium [37], and recently a source of pure granulocyte-CSF was described [41]. Both M-CSF and G-CSF need to be separated from the nonspecific inhibitory and stimulating proteins present in the crude preparations presently available. It should also be possible to prepare eosinophil-CSF (EO-CSF) from pokeweed-mitogen, spleen cell-conditioned medium [47].

METABOLIC EFFECTS OF HEMOPOIETIC REGULATORS

GM-CSF appears to stimulate quiescent fetal GM-progenitor cells into active DNA synthesis within 6 h [33]. The further observation that bone marrow-derived GM-clones stop proliferating when removed from GM-CSF [17] implies that GM-CSF also stimulates the more mature cells along the GM-differentiation pathway. GM-CSF also appears to stimulate macromolecular synthesis in the nondividing end cells of the differentiation pathway. After stimulation by GM-CSF for 6 h, bone marrow metamyelocytes and polymorphonuclear leukocytes synthesize RNA at almost twice the normal rate [60]. Similar experiments with bone marrow cells showed that this is followed by a concomitant increase in protein synthesis (after 9 h) and DNA synthesis (after 19 h) [57]. These increases were specific for cells of the granulocytic and monocytic series [60]; no stimulation of macromolecular synthesis was observed for nucleated erythroid or lymphocytic cells. GM-CSF from several mouse tumor cell lines also appear to stimulate mature macrophages to incorporate (^{3}H)-thymidine into DNA [55] and to release specific metabolites such as the prostaglandins [61]. Although the effects of GM-CSF on bone marrow cell RNA synthesis can be observed after 10 min [60], this increase may not be due to a uniform stimulation of all the cells in the granulocytic series. Similar studies on the stimulation of macromolecular synthesis have been performed for the action of erythropoietin on bone marrow and fetal liver cells [62–64]. Again the effects appear to be cell-specific, ie, erythroblasts are stimulated, but not other hemopoietic cells [65]. Erythropoietin also appears to stimulate the synthesis of specific globin mRNA in immature erythroblasts both in vitro and in vivo. The molecular mechanisms by which any of these regulators cause the changes either in gene expression [63] or macromolecular synthesis is not yet clear. Some experiments have indicated that the effects of GM-CSF and erythropoietin can be modulated by the cylic nucleotides [66, 67]. Similar experiments, however, failed to find any effect of cyclic AMP on the action of GM-CSF [68] or erythropoietin [62]. The effects of the cyclic nucleotides on the action of GM-CSF appeared to be on the cells producing the GM-CSF rather than the GM-progenitor cells [68]. A preliminary observation on the action of erythropoietin suggested that erythropoietin induced the production of a new cytoplasmic protein in bone marrow cells, which was able to stimulate RNA synthesis in the nuclei of cells from bone marrow, kidney, or lung [69]. This protein was not produced when kidney or lung cells were exposed to erythropoietin, nor did the new protein stimulate the RNA synthesis in whole cells [69]. Erythropoietin did not stimulate RNA synthesis of isolated bone marrow nuclei when mixed with the cytoplasmic proteins from unstimulated cells. Thus, there appeared to be the production of an intracellular protein with broad specificity as a result of the specific interaction of erythropoietin with its target cell. No direct

evidence has been obtained to test this mechanism of action for the other hemopoietic regulators.

HEMOPOIETIC CELL SURFACE

The surface proteins of hemopoietic cells presumably reflect the structural require-
ments of the cell membrane as well as functional specificities of a given cell type. A detailed
knowledge of the cell surface proteins should help our understanding of the relationships
between cells within a given differentiation sequence (eg, myeloblast, myelocyte and
polymorphonuclear granulocyte [32]) and the relationships between cells of different
hemopoietic lineage (eg, lymphocytes and macrophages). Many surface receptors are
shared by cells apparently only remotely related: For example, epithelial cells, eosinophils,
macrophages and granulocytes all possess Fc receptors for immunoglobulin [27, 70, 71].
Another interesting feature concerning the changes in the surface proteins of cells during
differentiation is the loss of particular proteins from the surface of hemopoietic progeni-
tors as differentiation proceeds along a specific hemopoietic pathway, whereas these same
proteins are retained on the surface of other mature cell types. Brain cells appear to share
surface proteins with the multipotential hemopoietic cell [72, 73], which are no longer
expressed on either mature bone marrow cells or the progenitor cells committed to gran-
ulocyte macrophage differentiation [74, 75]. Similarly, there appear to be proteins shared
by human B lymphocytes and committed GM-progenitor cells (Table I) that are not present
on metamyelocytes and granulocytes [30, 31]. The surface proteins detected antigenically
also appear to cross-react with viral proteins. A recent report indicates that an antiviral
serum cross-reacts with the multipotential hemopoietic cell and that this cross-reactivity
can be eliminated by absorption with xenotropic C-type virus [76].

The analysis of the surface proteins of different cell types is necessary for an under-
standing of the structural requirements of the cell membrane, the processing of signals
generated by receptor-ligand complexes, and the expression of specific cellular functions.
A detailed knowledge of the erythrocyte membrane is beginning to emerge, as well as the
characterization of many of the membrane proteins [77] and their function [78]; the
spatial relationships between some of these surface proteins and specific structural pro-
teins such as spectrin are now beginning to be understood [79]. The time-space relation-
ships of membrane proteins after stimulation and during cell division and differentiation
is thought to be important for the transmission of information across the cell membrane
[80] and may well be an essential part of cell-cell recognition during organogenesis [81].
There appears to be a direct linkage between the reorientation of membrane receptor
proteins and intracellular structural proteins [82] which could transmit information
directly from the surface to the nucleus [83]. Although there is some suggestion that the
reorientation of surface proteins may even be influenced by the cell nucleus, it appears
that fluid lipid bilayers generate spontaneously the aggregation of absorbed proteins in
response to partial cross-linking [84]. Hemopoietic cells are rapidly turning over their
membrane components by endocytosis [28], so that transmission of receptor, ligand
information almost certainly occurs by the internalization of whole portions of the cell
membrane [85] rather than by the indirect transmission of the information via secondary
channels.

The changes in the surface proteins of erythroid cells have been studied using
embryonic cells from the chick [86]. Erythroid cells which are homogeneous with regard
to their differentiation state are available from the circulation of the chick embryo at

various times of development. There are three proteins (MW 120,000, 45,000, and 29,000) present on immature erythroid cells which disappear rapidly during development. Conversely other cell surface proteins (eg, spectrin) and band 3 proteins are present at low levels in early erythroblasts but increase in their relative amounts with maturation [86]. There are many membrane proteins shared by the rabbit reticulocyte and erythrocyte; on the other hand there are particular proteins present on the reticulocyte membrane that disappear during maturation to mature erythrocytes [87, 88]. The proteins that appear on the cell surface are synthesized asynchronously during erythroid differentiation [88]. Quite possibly, functional or structural proteins are produced that are of importance only for directing the organization of the final array of proteins in the mature membrane; or in the case of the transferrin receptor [89] to allow the transport of metabolites necessary at a particular stage of erythroid differentiation. It will be interesting to see if it is possible to reassemble a functional membrane for a mature cell from its constitutents, or whether membrane proteins from the immature cells are necessary to allow the correct juxtaposition of the proteins for functional activity.

Although a considerable effort has been made to define the proteins of the lymphocyte membrane [25, 90, 91], many of those studies are hindered by the functional heterogeneity of the cell populations. Many functional and antigenic proteins exist on the lymphocyte membrane and the relationship of these receptors to the cytoplasmic infrastructure has generated considerable interest [82]. The membranes of other hemopoietic cells have not been studied extensively. It is known that complement component receptors [71], immunoglobulin receptors [71, and chemotactic receptors [26] exist on mature granulocytes and macrophages [32]. These receptors are not present on immature cells of the granulocyte macrophage series, but the receptors appear when these immature cells are stimulated nonspecifically, by means of thymopoietin or ubiquitin [92], or by more specific stimulation by GM-CSF [93]. A series of mouse myeloid tumor cells have been developed which appear to be blocked at specific stages of granulocyte macrophage differentiation [93]. Some of these tumor cells can be induced to differentiate to mature granulocytes with immunoglobulin and complement receptors using GM-CSF [93]. The mature cells also synthesize lysozyme but at a later stage of differentiation than the expression of the immunoglobulin or complement component receptors [95]. Other clones, while they respond to GM-CSF by an increased rate of proliferation, do not appear to differentiate morphologically [94]. The state of differentiation of these clones has not been defined as yet and this will require a more complete knowledge of the membrane structure of the immature cells along the granulocyte-macrophage differentiation pathway.

There is very little information about the mature of the cell surface proteins of even the mature granulocyte or macrophage membranes. This is somewhat surprising as these cells can be obtained in a high state of purity ($>$ 99%), and their functional role in the immunologic system is being studied intensely [96]. Our laboratory has started to study the mouse peritoneal granulocyte membrane proteins using external labeling [97]. The protein distribution appears to be remarkably simple (Fig 2) compared to the profiles of iodinated membrane proteins reported for mouse lymphocyte [90]. The major component labeled by 1, 3, 4, 6-tetrachloro-3α, 6α-diphenylglycoluril had an apparent mole molecular weight of 90,000 (Fig 2) when analyzed by SDS gel electrophoresis. Most of the proteins detected appear to have a molecular weight $>$ 50,000 (Fig 2), but if the level of iodination is increased, lower-molecular-weight components are observed. However, at these higher levels of iodination there appears to be significant labeling of actin, indicating that cytoplasmic as well as surface proteins are labeled. Surface labeling of granulocytes
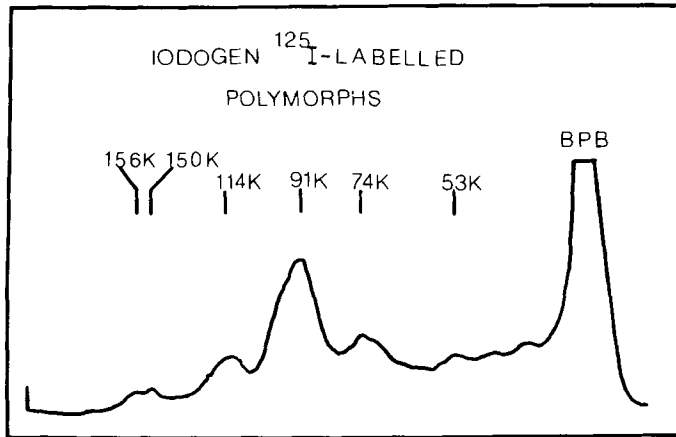
Fig 2. Analysis of ^{125}I-labeled surface proteins from mouse peritoneal polymorphonuclear neutrophils (PMN) using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. Iodogen was used to label the PMN cell surface with ^{125}I, and the membrane proteins were analyzed by electrophoresis on 8% polyacrylamide gels at pH 8.6 and subsequent autoradiography. The autoradiogram was scanned with a microdensitometer to determine the positions of the radiolabeled proteins. Molecular weight estimates were made by comparison to the relative mobilities of a set of standard proteins. (BPB = Bromophenol blue.)

and macrophages is complicated by the presence of intracellular myeloperoxidases, many endogenous proteases, and endocytosis. Particular care must be taken to avoid artifacts caused by proteolysis or intracellular labeling by the endogenous myeloperoxidase. Preliminary reports of the macrophage membrane proteins indicate that the major surface protein has a molecular weight of 90,000 [98]. It will be interesting to compare the surface proteins of granulocytes generated in vitro by stimulation of bone marrow progenitor cells using GM-CSF or M-CSF with the granulocytes isolated directly from the peritoneal cavity.

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