P. monacha, upon which it depends for sperm. Theoretically, unisexual individuals produce two female offspring for each one produced by a sexual individual, but this reproductive advantage is offset by a strong mating preference on the part of *P. monacha* males for conspecific females (*20*). Mathematical models were developed that showed how the interplay of these counteracting forces could permit a dynamic coexistence between the sexual host and its unisexual parasite (*21*). These models are unrealistic, however, because they assume that *P. 2 monacha-lucida* and *P. monacha* compete directly for limiting resources. Although females of the two forms may compete for sperm when males of *P. monacha* are rare, the unisexual and sexual females differ considerably in their utilization of food resources. Furthermore, it is erroneous to treat *P. 2 monacha-lucida* as if it were a single ecological entity. Although the exact origins of clones 1 and 2 are unknown, they are genetically and ecologically distinct. Nevertheless, from the present findings it is apparent that their continued coexistence, with one another and with *P. monacha*, depends in part on their ability to differentially exploit food resources in the heterogeneous environments of these desert streams.

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Limitation of Excessive Myelopoiesis by the Intrinsic Modulation of Macrophage-Derived Prostaglandin E

Abstract. *The clonal proliferation of the committed granulocyte-macrophage stem cell is controlled by a balance between mutually opposing factors, colony stimulating factor and prostaglandin E, both of monocyte-macrophage derivation. Increases beyond a critical concentration of colony stimulating factor within the local milieu of the mononuclear phagocyte induces the coincident elaboration of prostaglandin E, a self-regulated response which serves to limit the unopposed humoral stimulation of myelopoiesis.*

Simple cloning methods in semisolid medium which permit the selective in vitro proliferation of a particular population of committed stem cells have facilitated investigations into the cellular and molecular events controlling the proliferation and differentiation of hematopoietic cells. The bipotentially committed granulocyte-macrophage stem cell (colony forming unit-culture, CFU-C) can be detected by its ability to undergo clonal proliferation in soft agar medium when provided with stimulatory macromolecules (colony stimulating factor; CSF) (*1, 2*). Colony stimulating factor is active in vitro at extremely low concentrations (*2*) and does not stimulate the growth of other hematopoietic and nonhematopoietic cells (*3, 4*). Since CSF is detected in normal serums (*5*) and is generally increased in situations where there is increased granulopoiesis and monocyte-macrophage production (*3*), the humoral regulatory role of CSF in vivo may be analogous to that of erythropoietin. Thus, after the injection of antigens and bacterial endotoxin, during acute viral and bacterial infections, as well as preceding and during active myelopoietic regeneration following sublethal irradiation or treatment with cyclophosphamide, serum CSF concentrations are markedly increased (*6, 7*). Conversely, serum CSF levels are lower in germfree mice in which granulopoiesis is subnormal (*7, 8*). The principal CSF-producing cells, which have been identified as the blood monocyte and tissue macrophage (*9–11*), retain the ability to respond in vitro to endotoxin and markedly increase their production and release of CSF (*11*). Thus, granulopoiesis and monocyte-macrophage formation may be stimulated by a positive feedback mechanism involving CSF, which if not otherwise limited may result in accelerated myelopoiesis and further recruitment of CSF-producing cells. We reported previously (*4*) that the stimulatory actions of CSF on the committed stem cell CFU-C can be effectively limited by the synthetic E-series prostaglandins (PGE_1 and PGE_2). Conversely, increasing CSF concentrations counteract the PGE-mediated inhibition of CFU-C (*4*), indicating a dualistic modulation of committed stem cell proliferation. Here we extend these earlier, solely pharmacological observations of the CSF-PGE dualism into a self-regulating model of granulopoiesis and monocyte-macrophage production, which is controlled by the mononuclear phagocyte.

Soft agar cultures of normal human bone marrow cells were prepared as described (*4, 10*). Briefly, normal human bone marrow was separated on the basis of differential buoyant cell density by centrifugation in bovine serum albumin (density 1.070 g/cm^3) and active adherence to plastic culture dishes. The light density (< 1.070 g/cm^3) and nonadherent cells were suspended at a nucleated cell concentration of 1.5×10^5 cells per milliliter in McCoy's 5A modified medium containing 0.3 percent Bacto agar and supplemented with 15 percent fetal calf serum, essential and nonessential amino acids, vitamins, and sodium pyruvate. The bone marrow cell–agar suspensions were dispensed into tissue culture dishes (35 mm in diameter) and allowed to gel. After 10 days of incubation at 37°C in a humidified atmosphere of 10 percent CO_2 in air, the dishes were scored for the presence of colonies containing greater

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than 40 cells under × 25 magnification. The cultures were stimulated by the presence of various numbers of adherent human peripheral blood monocytes (density < 1.070 g/cm³). Direct physical contact between the monocytes and overlying bone marrow suspensions was prevented by an intervening 1-ml layer of 0.5 percent agar. In this manner, only diffusible interactions between monocytes and CFU-C were permitted.

The influence of diffusible monocyte-derived CSF on the clonal proliferation of human CFU-C is shown in Fig. 1. In those cultures containing a monocyte-free underlayer, no colony formation was observed, but in the presence of increasing numbers of adherent monocytes CFU-C were induced to proliferate (Fig. 1A). Above a monocyte concentration of 2.3×10^5 monocytes per culture, no further increase in net colony formation was observed. Addition of the prostaglandin (PGE) synthetase inhibitor indomethacin to the monocyte underlayers resulted in a linear stimulation of CFU-C proliferation over the concentration range of monocytes tested, and no diminution of colony formation was observed at high monocyte numbers. Indomethacin had no direct effect on CFU-C and did not stimulate colony formation in the absence of monocytes. The potentiating effect of indomethacin occurred only in the presence of appreciable numbers of monocytes ($\geq 2.3 \times 10^5$ per culture). In the absence of indomethacin, these numbers of monocytes released significant amounts of PGE in the medium of replicate cultures as determined by radioimmunoassay (Fig. 1B). The PGE first became detectable at a concentration of 2.3×10^5 monocytes, the optimal monocyte number above which no further increase in CFU-C stimulation occurred. The concentrations of PGE continued to increase as a linear function of the monocyte concentration. The inability of monocytes to synthesize PGE in the presence of indomethacin, permitted colony formation to occur solely in proportion to the underlying adherent monocyte population. Similar results have been obtained with murine marrow CFU-C and murine peritoneal macrophages. In this regard, cell separation procedures have shown that the principal PGE-producing cells in human peripheral blood and mouse peritoneal exudates are the monocytes and macrophages of light density that adhere to the culture dishes and possess histochemical affinity for α-naphthyl acetate esterase (*12*) and neutral red (*13*). In other experiments, prior treatment of adherent murine peritoneal cells with antiserum to theta and with complement had no effect on PGE production or inhibition of bone marrow CFU-C. Furthermore, PGE is undetectable in culture supernatants prepared from the nonadherent components of mouse peritoneal exudates, as well as from pure populations of normal human peripheral blood granulocytes (nonadherent, density > 1.070 g/cm³) and lymphocytes (nonadherent, density < 1.070 g/cm³) (*14*).

These findings indicate that the proliferation of the committed granulocyte-macrophage stem cell is regulated by both positive and negative feedback controls, involving monocyte-derived CSF and PGE, respectively. The accumulation of monocyte-derived PGE limits the numbers of CFU-C which undergo proliferation in response to a given concentration of monocyte-derived CSF and therefore results in an underestimate of the incidence of potentially clonable committed stem cells. By inhibiting the constitutive synthesis of PGE, the magnitude of CFU-C proliferation is determined solely by the net level of CSF which increases as a linear function of the monocyte concentration.

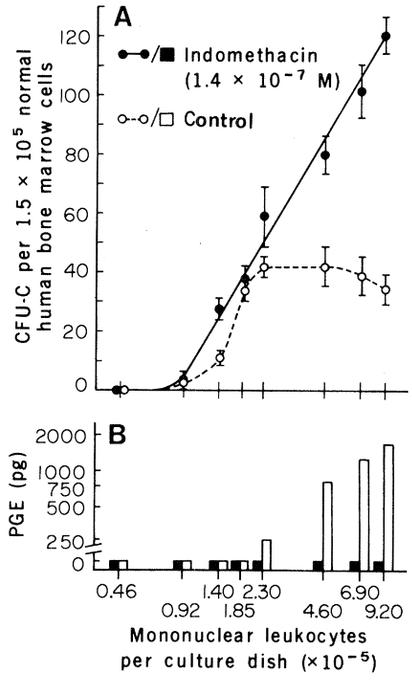

Fig. 1. Modulation human monocyte production of CSF and PGE by indomethacin. (A) Various numbers of adherent human mononuclear leukocytes (density < 1.070 g/cm³) were overlayed with 1.0 ml of 0.5 percent agar medium in the absence or presence of $1.4 \times 10^{-7}M$ indomethacin. Target cell suspensions (1 ml) of 1.5×10^5 normal human bone marrow cells in 0.3 percent soft agar medium were added above the adherent monocyte underlayers and the number of colonies containing greater than 40 cells in the bone marrow overlayers was determined after 10 days of culture. Quadruplicate cultures were scored for each point, and the results are expressed as the number of CFU-C (± standard error of the mean) which commenced proliferation to form colonies. (B) Replicate liquid cultures containing the identical number of adherent monocytes as in the agar underlayers were used to obtain supernatant media for determination of PGE production for each monocyte concentration. Measurements were obtained by radioimmunoassay (*20*).

We also investigated the homeostatic potential of the monocyte's effector role in hematopoiesis in terms of (i) the ability to increase its elaboration of a diffusible inhibitor of CFU-C proliferation in response to increasing concentrations of CSF, and (ii) whether this inhibition correlated with an increase in the elaboration of PGE by the mononuclear phagocyte. The species specificity of CSF action, whereby CSF of murine cell origin does not stimulate colony formation by human CFU-C (*1*), provided the basis of these investigations in which we utilized normal human bone marrow cells incorporated in soft agar cultures as the source of target-committed stem cells (Fig. 2). Such cultures were stimulated by the presence of human CSF elaborated by 1×10^6 normal human peripheral blood leukocytes suspended in a 0.5 percent agar underlayer, and the numbers of CFU-C which underwent clonal proliferation to form colonies is shown by the shaded area in Fig. 2A. Cell-free supernatants from 48-hour liquid cultures of 1×10^6 murine peritoneal macrophages per milliliter were prepared in the absence and presence of a potent source of murine CSF, provided by conditioned medium from a murine myelomonocytic leukemic cell line (WEHI-3) (*4*). Neither the individual presence of the murine macrophage supernatant, nor the murine CSF to which the macrophages were exposed, had any effect on the human CSF-stimulated CFU-C proliferation when tested at 10 percent by volume in soft agar cultures. However, the peritoneal macrophage supernatant prepared in the continued presence of the murine CSF profoundly inhibited the proliferation of human CFU-C stimulated by the human leukocyte-derived CSF (Fig. 2A). The magnitude of human CFU-C inhibition was proportional to the concentration of murine CSF present during the active generation of the murine macrophage supernatant. After simple dialysis, these same macrophage supernatants lost all detectable inhibitory activity against human bone marrow CFU-C. These findings indicate that murine macrophages, which have other-

wise no effect on exogenously stimulated human CFU-C, are induced by increasing concentrations of murine CSF to elaborate a low molecular weight, nonspecies-specific inhibitor of committed granulocyte-macrophage stem cell proliferation.

As shown in Fig. 2B, the same concentrations of murine CSF which promoted the elaboration of CFU-C inhibitory factor, induced the coincident production of PGE by 1×10^5 murine peritoneal macrophages incubated under conditions identical to the preparation of the macrophage supernatants used in the previous experiment (*15*). Note the inverse relation between the CSF-stimulated levels of PGE (Fig. 2B) and the CSF-induced human CFU-C inhibitory activity (Fig. 2A), both represented as the constitutive contribution of 1×10^5 macrophages. In the absence of PGE production, the diffusible contribution of 1×10^5 murine macrophages are entirely without CFU-C inhibitory activity. More striking is the manner by which small increases in the CSF-induced production of PGE by macrophages can account for the marked diminution in the numbers of proliferating CFU-C (*16, 17*).

The present findings are based on techniques available for assaying hematopoietic cell cloning in vitro, but are sufficient to suggest physiological relevance to the situation in vivo. In this regard, the mononuclear phagocyte is implicated as the central cellular element in the humoral control of granulopoiesis and monocyte-macrophage production. The ability of this cell population to elaborate both CSF and PGE, agents which exert an opposing proliferative influence on the committed granulocyte-macrophage stem cell (CFU-C), indicates that this regulation is based on a balance of both positive and negative feedback mechanisms. This model explains some of the opposing effects of the mononuclear phagocyte on hematopoietic cell proliferation.

Under basal conditions, appropriate levels of CSF elaborated by the monocyte-macrophage population (*9–11*) stimulate the committed stem cell to commence proliferation. The constitutive contribution of CSF to granulopoiesis and monocyte-macrophage production is rapidly increased under physiologically perturbed circumstances. Thus, after trauma or during acute viral or bacterial infections, increases in both systemic and local CSF concentrations rapidly ensue (*6, 7, 11*). This increased CSF stimulates a greater number of stem cells to proliferate which, in addition to recruiting granulocytes, results in an expansion of the population of CSF-producing mononuclear phagocytes. Such a positive feedback control of myelopoiesis is ultimately restricted by the synthesis and release of PGE by the mononuclear phagocyte components of the myeloid clone. Just as CSF promotes continued replication of the CFU-C and its progeny, PGE limits this effect by an opposing action on the responsiveness of the myeloid stem cell and proliferating progeny to stimulation by CSF. Thus, the net proliferative potential of the myelopoietic clone is determined by the relative levels of CSF and PGE, which in turn is controlled by a balance between both positive and negative feedback mechanisms of the mononuclear phagocyte.

Since two opposing feedback mechanisms have their origins in a common regulatory cell, the two mutually antagonistic feedback events are probably causally associated. Such a proposition is substantiated by the observations that progressive increases in CSF beyond a critical concentration are ultimately sensed by the macrophage and serve to stimulate the coincident production and release of PGE. The concentrations of macrophage-derived PGE increase in parallel with the local CSF concentration, thereby implicating the mononuclear phagocyte as a surveillance cell which functions to maintain myelopoiesis within appropriate limits. The extreme lability of the prostaglandin molecule (*18*) may provide a further physiological control, for which the continued presence of an elevated CSF stimulus may be a requisite for the maintenance of a critical PGE concentration. In this regard, a diminution in the production of CSF by monocytes and macrophages incubated in the presence of an extract of mature polymorphonuclear leukocytes (*19*) results in a coincident decrease in the elaboration of PGE (*17*). Thus, the endogenous level of CSF may in part determine the ability of the mononuclear phagocyte to synthesize PGE.

The ability of the macrophage to control the proliferation of its own progenitor cell by both positive and negative feedback mechanisms has significant relevance to other cell renewal systems. Though the present observations have been defined in vitro, one may speculate that loss of the surveillance function by the mononuclear phagocyte, or the inability to maintain appropriate negative

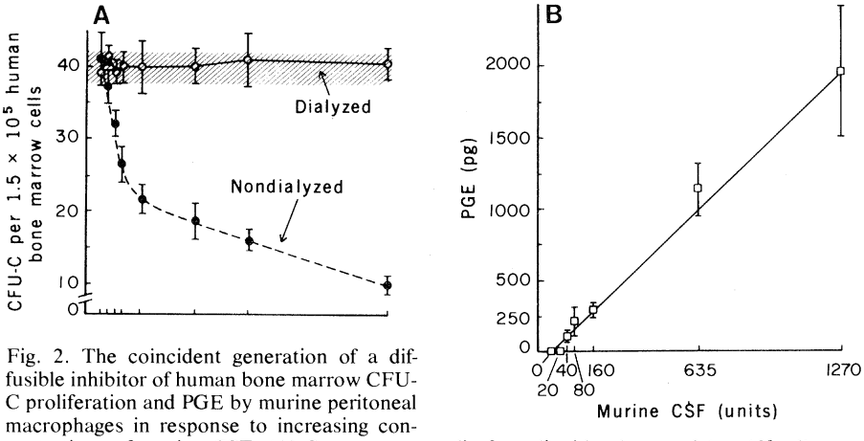

Fig. 2. The coincident generation of a diffusible inhibitor of human bone marrow CFU-C proliferation and PGE by murine peritoneal macrophages in response to increasing concentrations of murine CSF. (A) Supernatant media from liquid cultures of 1×10^6 adherent thioglycollate-induced B6D2F$_1$ peritoneal macrophages per milliliter incubated in the absence and presence of increasing concentrations of murine CSF (provided by WEHI-3 conditioned medium) were collected after 48 hours. Half of the murine macrophage supernatants were dialyzed against five changes of phosphate-buffered saline containing 5.0 percent fetal calf serum for 3 days, and sterilized by Millipore filtration (0.45 μm pore size); 0.1 ml of each dialyzed and nondialyzed sample was added to soft agar cultures of 1.5×10^5 normal human bone marrow cells (density < 1.070 g/cm^3). The human target cells were stimulated by underlying feeder layers of human leukocytes consisting of 1×10^6 leukocytes suspended in 1.0 ml of 0.5 percent agar medium, and the numbers of CFU-C which had undergone clonal proliferation in four replicate cultures to generate colonies were determined after 7 days of incubation. The shaded area indicates control colony formation by human bone marrow CFU-C (± standard error of the mean) in the presence of the leukocyte feeder layer. (B) Thioglycollate-induced B6D2F$_1$ peritoneal macrophages (1×10^5 per milliliter) were incubated in the absence or presence of murine CSF (WEHI-3 conditioned medium). After 48 hours the supernatants were harvested and analyzed by radioimmunoassay for PGE. The results are expressed as the mean concentration of PGE (± standard error of the mean) elaborated by 1×10^5 murine macrophages in four replicate cultures. Note that 1000 pg of PGE is equivalent to $2.86 \times 10^{-6} M$ PGE, a concentration of synthetic PGE which significantly inhibits CSF-stimulated mouse bone marrow cultures (*4*).

controls, may foster overzealous myelopoiesis in the face of unopposing stimulation. Thus, certain of the myeloproliferative disorders may represent a defect in macrophage-mediated feedback mechanisms.

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European Corn Borer: Pheromone Polymorphism or Sibling Species?

Abstract. *Electrophoretic analyses of the* (Z) *and* (E) *pheromone-attracted males of* Ostrinia nubilalis *(Hübner), the European corn borer, in an area of coexistence indicate that these strains are not freely interbreeding. Although the populations are morphologically indistinguishable, studies of allozyme, pheromone, and hybridization suggest that the* (Z) *and* (E) *entities are genetically differentiated, perhaps to the status of semi- or sibling species.*

Pheromone investigations of the European corn borer, *Ostrinia nubilalis* (Hübner) (*1*), have shown two distinct population types: the (*Z*) strain, producing and attracted to a 97:3 mix of (*Z*)-11- and (*E*)-11-tetradecenyl acetate, and the (*E*) strain, producing and attracted to a 4 : 96 blend of the same compounds (*2, 3*). The (*Z*) strain is widely distributed, representing nearly all the populations surveyed to date in Europe as well as those in North America (*4*), where this important agricultural pest was established by at least two separate introductions in the early 1900's (*5*). The (*E*) strain occurs allopatrically in Italy and New York (*3, 4*). The two strains occur sympatrically and synchronically in central Pennsylvania (*6*), stimulating speculation on the status of *O. nubilalis* as a monotypic species, the possibility of pheromone polymorphism, and the origin and ultimate fate of the strains in sympatry.

Allozyme data suggests genetic divergence between the (*Z*) and (*E*) strains. In Amity Hall, Pennsylvania, males were lured to synthetic blends, ensnared in sticky traps, removed live, and frozen until electrophoretic analyses (*7*). For this group of males attracted to either (*Z*) or (*E*) blends, allozyme frequencies at ten loci (Table 1) show that malate dehydrogenase (MDH-1), isocitrate dehydrogenase (IDH-1), α-glycerophosphate dehydrogenase (α-GPD), and 6-phosphogluconate dehydrogenase (6-PGD) were either monomorphic or had a second allele at a very low frequency. Of the polymorphic loci, glutamate oxaloacetate transaminase (GOT-1 and GOT-2), phosphohexose isomerase (PHI), and phosphoglucomutase (PGM) showed significant differences between strains ($P < .05$), whereas IDH-2 and MDH-2 were not statistically different. When these electrophoretic data are compared with the use of Nei's (*8*) index of genetic identity (*I*), the similarity between strains is 0.997. Values of *I* for sibling species generally range from 0.98 to less than 0.70 (*9*) with only a weak correlation with the degree of morphological identity (*10*). Genetic identity may be especially inadequate to separate species that have recently diverged, because only a few genes may be involved in the initial phase of speciation (*11*). Our data do not rule out the possibility of interbreeding, although if introgression is occurring, it should be of recent origin or of low magnitude.

Natural hybrids of the (*Z*) and (*E*) strains may not be at a survival disadvantage, because laboratory F_1, F_2, and backcross progeny exhibit heterosis. However, in laboratory mating choice tests under confined conditions (in which the necessity for long-distance mate location by pheromone was eliminated), the frequencies of successful interstrain crosses were 6 and 10 percent [for the ♂ (*Z*) × ♀ (*E*) and ♂ (*E*) × ♀ (*Z*) matings, respectively], whereas the frequencies of successful intrastrain control crosses were 80 and 76 percent for the (*Z*) and (*E*) classes. The average daily time of mating in the laboratory within a 24-hour cycle differed by 1.7 hours with considerable overlap, so that exclusive mating cycles alone would not seem to effectively isolate these strains (*12*).

In the field (Table 2), attraction to the various blends indicates yearly shifts in the relative proportions of the strains. In 1973 the two strains appeared to occur in similar numbers, while from 1974 to 1977

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