

Differential Interleukin-1 Elaboration by Density-Defined Human Monocyte Subpopulations

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Interleukin-1 (IL-1) is an important immunoregulatory peptide produced by monocytes and macrophages. Because mononuclear phagocytes are morphologically and functionally heterogeneous, we examined whether they differ in their ability to elaborate IL-1. We used discontinuous Percoll gradients to obtain five density-defined human blood monocyte subpopulations. Unfractionated monocytes and their subsets were compared for their ability to stimulate thymocyte proliferation. Supernatants obtained

from the denser monocytes consistently contained more IL-1 activity than did supernatants from the less dense cells. This difference in IL-1 activity was the result of differences in IL-1 elaboration, not the selective production of an inhibitor of IL-1-induced thymocyte proliferation. These data demonstrate that density-defined human monocyte subpopulations differ in their capacity to elaborate IL-1.

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INTERLEUKIN-1 (IL-1) is an important immunoregulatory monokine. Mononuclear phagocyte elaboration of IL-1 is important in T and B lymphocyte differentiation and proliferation, lymphokine elaboration, and antibody production.¹⁻⁵ In addition, IL-1 can stimulate hypothalamic cells to cause fever,⁶ hepatocytes to increase their elaboration of acute phase proteins,^{7,8} and fibroblasts to alter their growth, prostaglandin synthesis, and collagenase production.⁹⁻¹¹

Mononuclear phagocytes are morphologically and functionally heterogeneous. We have previously observed that density-defined human monocyte subpopulations differ in their Fc (IgG) and C3 receptor activity and in their capacity to support pokeweed mitogen-induced B cell differentiation.¹² In addition, density-defined human monocyte subpopulations differ in their ability to elaborate colony-stimulating activity¹³ and prostaglandins.¹⁴ These findings led us to speculate that density-defined monocytes might differ in their ability to elaborate IL-1. To test this hypothesis, human monocytes were separated into five density-defined subpopulations using discontinuous gradients of Percoll (Pharmacia, Piscataway, NJ). The IL-1 activity of supernatants obtained from unfractionated monocytes and these monocyte subpopulations were then compared. The data indicate that density-defined monocytes differ in their ability to elaborate IL-1, with denser cells producing more IL-1 than less dense cells.

MATERIALS AND METHODS

Monocyte preparation. Monocytes were isolated as previously described.¹⁵ Peripheral blood mononuclear cells were obtained from normal volunteers by Ficoll-Hypaque centrifugation (SG 1.077). The cells were then adhered to plastic in Dulbecco's modified Eagle's medium supplemented with nonessential amino acids, penicillin,

streptomycin, and 10% heat-inactivated fetal calf serum (FCS) (GIBCO, Grand Island, NY) (complete media). The flasks used in these studies were pretreated with FCS as previously described.¹⁶ After incubation for one hour at 37 °C in 5% CO₂ and air, the flasks were washed vigorously, and the nonadherent cells were discarded. The adherent cells were then mechanically detached and washed thoroughly. The resultant cell suspension was ≥95% monocytes as assessed by light microscopy after staining with modified Giemsa (Diff Quik, Harleco, Philadelphia) and nonspecific esterase stains.¹⁷

Monocyte subpopulation preparation. The monocytes were fractionated into five density-defined subpopulations, using discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradients as previously described.¹⁵ Monocytes (2×10^7) were layered onto a 10-mL gradient containing 2 mL each of Percoll, SG 1.045, 1.050, 1.055, 1.060, and 1.065. The gradients were then centrifuged in a Sorval RT-6000 (DuPont Institute, Wilmington, Del) for ten minutes at room temperature (450 g). The cells that localized at each gradient interface were harvested, washed, and resuspended. Viability was >90% by trypan blue dye exclusion, and recovery varied from 60% to 80%. The different monocyte subpopulations were designated as numbers 1 through 5. The cells in fraction 1 were the least dense (SG ≤ 1.045), and the cells in fraction 5 were the most dense (SG between 1.060 and 1.065).

Supernatant preparation. Unfractionated and fractionated monocytes (5×10^5 /mL) were suspended in round-bottom microtiter wells (Limbro, Hamden, Conn) in complete medium in the presence or absence of 50 µg/mL of lipopolysaccharide (LPS) (Difco, Detroit). After incubation for 24 hours at 37 °C in 5% CO₂ and air, the individual wells were pooled and the cell-free supernatants were obtained after centrifugation (400 g for 15 minutes).

Assay of IL-1 activity. The IL-1 activity of our supernatants was assessed by standard mouse thymocyte assay as described by Mizel.¹⁸ Thymocytes were obtained from three- to six-week-old C3H/HEJ mice and incubated with dilutions of control medium or test supernatants (1.5×10^6 thymocytes per well). Proliferation was assessed with or without a suboptimal dose (10 µg/mL) of phytohemagglutinin (PHA) (Difco). The cultures were then incubated at 37 °C in 5% CO₂ and air for 72 hours. Sixteen hours before the end of the incubation period, thymocyte proliferation was assessed by labeling with 0.5 µCi ³HTdr (5 ci/mmol, Amersham, Arlington Heights, Ill). At the end of the incubation period, the cells were harvested, and cellular ³HTdr incorporation was assessed by scintillation counting.

Assay of IL-2 activity. To determine whether our supernatants contained IL-2, we assayed for its presence by using techniques previously described.¹⁹ In brief, blood mononuclear cells were cultured with PHA for four days and then in the presence of IL-2 containing supernatants for an additional ten days. After this ten-day period, these cells (designated continuous T cells [CTC]) have an absolute requirement for IL-2 for continued proliferation.

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The ability of monocyte supernatants to maintain the proliferation of these cells was compared with that of supernatants containing IL-2.

Statistics. Intraexperimental differences between the mean values for a set of observations were assessed by the use of the standard Student's *t* test. Comparisons between the IL-1 activities in supernatants from the different monocyte populations were performed with nonparametric analysis (Friedman Free Rank Order).²⁰

RESULTS

Unfractionated blood monocytes. Supernatants from unstimulated blood monocytes contained either trace or undetectable amounts of IL-1 activity. In contrast, supernatants from LPS-stimulated blood monocytes contained large amounts of IL-1 activity, briskly stimulating thymocyte proliferation (Table 1). The effect was dose-dependent, with peak thymocyte proliferation occurring in wells containing a 1:4 dilution of supernatant. The majority of this stimulatory activity fractionated at a molecular weight between 14,000 and 16,000 daltons, using gel filtration techniques previously described.¹³ In addition, these supernatants consistently contained no IL-2 activity when tested for their ability to support the proliferation of continuous T cell lines.

Percoll-fractionated cells. Fractionation using a discontinuous Percoll gradient resulted in five monocyte subpopulations. Recovered cells were >98% monocytes and were >90% viable, as were the unfractionated cells. Supernatants from unstimulated monocytes from all fractions contained trace or no IL-1 activity. Supernatants from LPS-stimulated monocytes from all fractions were capable of stimulating thymocyte proliferation. However, the IL-1 activity of supernatants from the density-defined monocyte subpopulations differed significantly. Supernatants from the dense monocytes (fractions 3, 4, and 5) were consistently more potent stimulators of thymocyte proliferation than supernatants from the less dense cells (fractions 1 and 2) (Table 1, Fig 1). Overall, supernatants from fraction 4 cells were the most potent, frequently causing thymocytes to incorporate more ³HTdr than did supernatants from the unfractionated cells. Lesser amounts of IL-1 activity were found in supernatants from cells from fractions 3 and 5. In contrast, supernatants from monocytes in fractions 1 and 2 contained the least IL-1 activity (Table 1, Fig 1). These supernatants frequently caused less thymocyte proliferation than supernatants from the unfractionated cells (Fig 1). These differences in IL-1 activity were seen at all dilutions of supernatant tested (1:4 to 1:16). In addition, dialysis against complete medium using a 12,000 dalton molecular weight cutoff membrane did not alter the pattern of IL-1 activity in

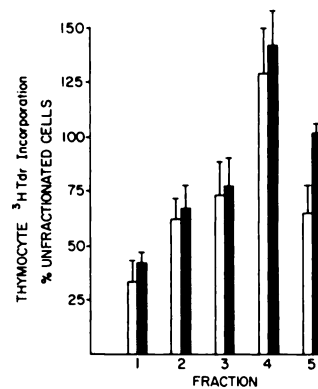


Fig 1. Cumulative illustration of the effect on thymocyte ³HTdr incorporation of a 1:8 dilution of supernatants from LPS-stimulated unfractionated and fractionated blood monocytes. The ³HTdr incorporated by thymocytes incubated in supernatants from unfractionated cells. The IL-1 activity in the monocyte supernatants was assessed in the presence (■) and absence (□) of suboptimal concentrations of PHA. Each value represents the mean ± SE of eight experiments in which all monocyte subpopulations were studied.

the supernatants from the density-defined monocyte subpopulations. The differences in IL-1 activity in supernatants from fx 4 cells and the activity in supernatants from fx 1 and fx 2 monocytes were significant at the *P* ≤ .009 level using nonparametric analysis.

The noted differences in IL-1 activity could result from variation in the rate of synthesis of IL-1 or from the selective elaboration by the less dense monocytes of a factor(s) that interferes with IL-1-induced thymocyte proliferation. To see if such an inhibitor was being selectively elaborated, mixing experiments were performed in which thymocytes were incubated with IL-1 containing control blood monocyte supernatants with and without the addition of dilutions of supernatants from all density-defined monocyte subpopulations. Supernatants from fraction 1 and fraction 4 cells had similar effects on thymocyte proliferation in this assay (data not shown). Thus, the differences in IL-1 activity that were noted were not caused by the selective elaboration of an inhibitor of IL-1-induced thymocyte proliferation.

Monocyte fractions 1 and 2 contained 5.3% and 5.2%, respectively, of all recovered cells. In contrast, fractions 3, 4, and 5 contained 9.9%, 36.8%, and 42.5%, respectively, of all recovered cells (Fig 2). Thus, 89.2% of all recovered monocytes were in fractions 3 through 5, the monocytes that were

Table 1. Interleukin-1 Activity of Unfractionated and Density-Defined Monocyte Subpopulations

Experiment No.	Thymocyte ³ HTdr Incorporation (cpm)					
	Unfractionated	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5
1	9,331 ± 414	1,138 ± 234	1,897 ± 303	3,140 ± 459	8,694 ± 427	5,449 ± 92
2	5,484 ± 284	1,458 ± 202	1,579 ± 156	2,269 ± 82	6,295 ± 198	3,980 ± 391
3	11,122 ± 1,075	4,576 ± 502	5,860 ± 540	7,956 ± 801	10,596 ± 707	6,224 ± 501
4	7,588 ± 658	3,223 ± 401	6,157 ± 576	9,727 ± 1,078	11,229 ± 1,004	5,841 ± 575

Representative experiments (n = 12) illustrating the effect on thymocyte ³HTdr incorporation of 1:8 dilution of supernatants from LPS-stimulated unfractionated monocytes and density-defined (Percoll) monocyte subpopulations. Thymocytes cultured without monocyte supernatants consistently incorporated less than 500 cpm. All cultures contained 10 µg/mL of PHA.

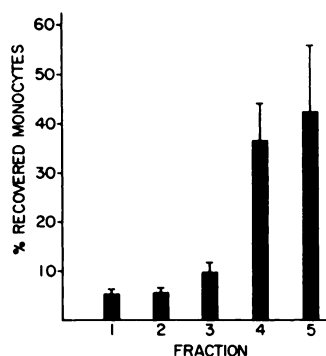


Fig 2. Density distribution of blood monocytes recovered from the Percoll gradients. The bars represent the percentage of all cells recovered at each density level. Values represent the mean \pm SEM of nine experiments.

efficient producers of IL-1. Conversely, 10.8% of all recovered cells were in fractions 1 and 2, monocytes that were inefficient producers of IL-1. These findings suggest that the majority of circulating blood monocytes are capable of elaborating significant amounts of IL-1 on stimulation.

DISCUSSION

In an effort to further understand the importance of monocyte and macrophage heterogeneity in the immune response, we characterized the IL-1 activity of supernatants obtained from density-defined monocyte subpopulations. Our data indicate that supernatants from denser monocytes stimulate thymocyte proliferation to a greater degree than do supernatants from less dense cells. In addition, we demonstrate that the differences in IL-1 activity are the result of differences in IL-1 elaboration rather than the selective production of an inhibitor of IL-1-induced thymocyte proliferation. The major thymocyte-stimulating activity in our supernatants had an apparent molecular weight between 14,000 and 16,000 daltons. In addition, our supernatants did not support the continued proliferation of IL-2-dependent continuous T cell lines. Thus, we feel that the activity being assayed is truly that of IL-1. However, the small number of cells available from a number of the density-defined subpopulations precludes further characterization at present.

Separation of monocytes on the basis of density has previously proven useful for investigating differences among mononuclear phagocytes. Density-defined human monocytes have been shown to differ in their esterase activity,¹³ capacity to yield colony-stimulating activity,¹³ prostaglandin E₂ production,¹⁴ Fc(IgG) and C3 receptor activity,¹² and in their ability to support pokeweed mitogen-induced B cell differentiation.¹² Our studies show that density-defined human monocytes also differ in their ability to elaborate IL-1. This finding in humans is in accord with the prior demonstration by Shellito and Kaltreider that density-defined rat alveolar macrophage subpopulations differ in their ability to elaborate IL-1 after stimulation with phorbol myristate acetate.²¹ In addition, the finding that monocytes of greater density are more efficient producers of IL-1 than are monocytes of lesser density concurs with prior studies in humans and animals, in

whom more dense mononuclear phagocytes exhibited enhanced functional activity. Shellito and Kaltreider observed that denser rat alveolar macrophages were more potent producers of IL-1.²¹ We previously demonstrated that denser human monocytes express more Fc (IgG) and C3 receptor activity and are enriched in their capacity to support pokeweed mitogen-induced B cell differentiation.¹² Zwilling et al showed that denser hamster alveolar macrophages were more efficient killers of tumor cells after exposure to stimulated lymphocyte supernatants,²² and Holian et al showed that denser guinea pig alveolar macrophages exhibited increased stimulated migration, superoxide anion release, and pinocytosis.²³ In addition, Serio et al showed that denser mouse peritoneal macrophages were more efficient mediators of antibody-dependent phagocytosis and had enhanced Fc (IgG) receptor activity,²⁴ whereas Murphy et al showed that denser rabbit alveolar macrophages augmented antigen-stimulated lymphocyte proliferation to a greater degree than did less dense cells.²⁵

It is not clear if the differences in IL-1 elaboration by fractionated monocytes reflect different capabilities of cells derived from different precursor pools or different capacities of cells derived from the same precursors at different stages of maturation or activation. In an attempt to gather information in this regard, we characterized the ability of monocytes to elaborate IL-1 as they mature in vitro. We noted that after 48 to 72 hours in culture, monocytes have a diminished capacity to elaborate IL-1 in response to LPS (J.A.E. and A.D.S., unpublished observation, April 1985). This observation is in accord with reports from others.^{26,27} Thus, a monocyte's capacity to elaborate IL-1 can be altered by time and/or environment and is not fixed on the basis of the precursor cell of origin. This observation is in accord with the hypothesis that the differential IL-1 production described in this report is a function of cell maturation or activation. However, further experimentation will be required to definitively prove this hypothesis.

Our data demonstrate that density-defined monocyte subpopulations differ in their ability to elaborate IL-1. If density-defined human tissue macrophages also differ in their ability to elaborate IL-1, this finding has a number of important potential implications. IL-1 can have important effects on T and B lymphocyte proliferation and on differentiation, lymphokine elaboration, and antibody production.¹⁻⁵ IL-1 may also affect hypothalamic cells, causing fever⁶; hepatocytes, increasing the elaboration of acute phase reactants^{7,8}; and fibroblasts, altering their growth and increasing their prostaglandin and collagenase production.⁹⁻¹¹ Thus, the relative distribution of mononuclear phagocytes may affect the amount of IL-1 elaborated by a mononuclear phagocyte population and thus the intensity of local inflammatory and fibrotic processes.

In summary, we have shown that density-defined human blood monocyte subpopulations differ in their ability to elaborate IL-1. We have also shown that the majority of circulating blood monocytes are efficient producers of IL-1. These findings further support the contention that human mononuclear phagocytes are a functionally heterogeneous cell population.

REFERENCES

1. Kampschmidt RF: The numerous postulated biological manifestations of interleukin-1. *J Leukocyte Biol* 36:341, 1984
2. Mizel SB: Interleukin-1 and T cell activation. *Immunol Rev* 63:51, 1982
3. Maizel AL, Mehta S, Ford RJ, Lachman LB: Effect of IL-1 on human thymocytes and purified human T cells. *J Exp Med* 153:470, 1981
4. Lipskey PE, Thompson PA, Rosenwasser LJ, Dinarello CA: The role of interleukin-1 in human B cell activation: Inhibition of B cell proliferation and the generation of immunoglobulin secreting cells by an antibody against human leukocytic pyrogen. *J Immunol* 130:2708, 1983
5. Thiele DL, Lipskey PE: The accessory function of phagocytic cells in human T cell and B cell responses. *J Immunol* 129:1033, 1982
6. Rosenwasser LJ, Dinarello CA: Ability of leukocyte pyrogen to enhance murine thymocyte proliferation. *Cell Immunol* 63:134, 1981
7. Sipe JD, Vogel SN, Ryan JL, McAdam KPWJ, Rosenstreich DL: Detection of a mediator from endotoxin stimulated macrophages that stimulates the acute phase serum amyloid A response in mice. *J Exp Med* 150:597, 1979
8. Sipe JD, Vogel SN, Szein MD, Skinner M, Cohen AS: The role of interleukin-1 in acute phase serum amyloid A (SAA) and serum amyloid P (SAP) biosynthesis. *Ann NY Acad Sci* 389:137, 1982
9. Mizel SB, Dayer JM, Krane SM, Mergenhagen SE: Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte activating factor (interleukin-1). *Proc Natl Acad Sci USA* 78:2474, 1981
10. Dayer JM, Goldring SR, Robinson DR, Krane SM: Effects of human mononuclear cell factor on cultured rheumatoid synovial cells. Interactions of prostaglandin E₂ and cyclic adenosine 3'5'monophosphate. *Biochim Biophys Acta* 586:87, 1979
11. Schmidt JA, Oliver CN, Lepe-Zuniga JL, Green I, Gery I: Silica stimulated monocytes release fibroblast proliferation factors identical to interleukin-1: A potential role for interleukin-1 in the pathogenesis of silicosis. *J Clin Invest* 73:1462, 1984
12. Schreiber AD, Kelley M, Dziarski A, Levinson AI: Human monocyte functional heterogeneity: Monocyte fractionation by discontinuous albumin gradient centrifugation. *Immunology* 49:231, 1983
13. Tice D, Goldberg J, Nelson DA: Functional properties of isopycnic fractions of human peripheral blood monocytes. *J Reticuloendothel Soc* 29:459, 1981
14. Goldyne ME, Stobo JD: Synthesis of prostaglandin by subpopulations of human peripheral blood monocytes. *Prostaglandins* 18:687, 1979
15. Elias JA, Zurier RB, Schreiber AD, Leff JA, Daniele RP: Monocyte inhibition of lung fibroblast growth: Relationship to fibroblast prostaglandin production and density defined monocyte subpopulations. *J Leukocyte Biol* 37:15, 1985
16. Kumagai K, Itoh K, Hinman S, Tada M: Pretreatment of plastic petrie dishes with fetal calf serum. A simple method for macrophage isolation. *J Immunol Methods* 29:17, 1979
17. Yam LT, Li CY, Crosby WH: Cytochemical identification of monocytes and granulocytes. *Am J Clin Pathol* 55:283, 1971
18. Mizel SB: Production and quantitation of lymphocyte activating factor (interleukin-1), in Herscovitz HB, Holden HT, Belanti JA, Ghaffar A (eds): *Manual of Macrophage Methodology*. New York, Marcel Dekker, 1981, pp 423-429
19. Koretzky GA, Daniele RP, Nowell PC: A phorbol ester (TPA) can replace macrophages in human lymphocyte cultures stimulated with mitogen but not with an antigen. *J Immunol* 128:1776, 1982
20. Hollander M, Wolfe DA: *Non-parametric Statistical Methods*. New York, Wiley, 1973
21. Shellito J, Kaltreider HB: Heterogeneity of immunologic function among subfractions of normal rat alveolar macrophages. *Am Rev Res Dis* 129:747, 1984
22. Zwilling BS, Campolito LA, Reiches NA: Alveolar macrophage subpopulations identified by differential centrifugation on a discontinuous albumin gradient. *Am Rev Res Dis* 125:448, 1982
23. Holian A, Dauber JH, Diamond MS, Daniele RP: Separation of bronchoalveolar cells from the guinea pig on continuous gradients of Percoll: Functional properties of fractionated lung macrophages. *J Reticuloendothel Soc* 33:157, 1983
24. Serio C, Gandour DM, Walker WS: Macrophage functional heterogeneity: Evidence for different antibody dependent effector cell activities and expression of Fc receptors among macrophage subpopulations. *J Reticuloendothel Soc* 25:197, 1979
25. Murphy MA, Herscovitz HB: Heterogeneity among alveolar macrophages in humoral and cell mediated immune responses: Separation of functional subpopulations by density gradient centrifugation on Percoll. *J Leukocyte Biol* 35:39, 1984
26. Mayernik DG, Haq A, Rinehart JJ: Interleukin-1 secretion by human monocytes and macrophages. *J Leukocyte Biol* 36:551, 1984
27. Rosenberg SA, Lipsky PE: The role of monocytes in pokeweed mitogen stimulated human B cell activation: Separate requirements for intact monocytes and a soluble monocyte factor. *J Immunol* 126:1341, 1981