Histiocytosis X

Purified ($T6^+$) Cells from Bone Granuloma Produce Interleukin 1 and Prostaglandin E_2 in Culture

F. Arenzana-Seisdedos, S. Barbey, J. L. Virelizier, M. Kornprobst, and C. Nezelof
Unité d'Immunologie et Rhumatologie Pédiatrique and Groupe de Pathologie Pédiatrique, INSERM U 132, Hôpital Necker Enfants Malades, 75743 Paris Cedex 15, France; Centre National de Transfusion Sanguine, Hôpital Saint Antoine, 184, Faubourg Saint Antoine, 75571 Paris Cedex 12, France

Abstract

We have investigated the secretory function of cell suspensions from bone eosinophilic granulomas surgically collected in two patients with histiocytosis X. Unseparated cell preparations spontaneously produced interleukin 1 (IL-1) and prostaglandin E_2 (PGE_2). In order to ascertain that this secretion was due to the characteristic Langerhans cell-like histiocytosis X cells predominantly found in the bone lesions, we have purified $T6^+$ cells by the use of a fluorescence-activated cell sorter. Such highly purified cell preparations were found to secrete IL-1 and PGE_2 spontaneously in culture. Stimulation with endotoxins and treatment with interferon gamma (IFN γ) revealed an intense IL-1 secretory function of histiocytosis X cells. Since both IL-1 and PGE_2 are able to induce bone resorption in vitro, our findings are compatible with the hypothesis that histiocytosis X cells are responsible for the typical osteolytic lesion observed in histiocytosis X through the local secretion of these two mediators.

Introduction

Among the syndromes regrouped under the term histiocytosis X (1), eosinophilic granuloma is the more frequent clinical and histological entity (2, 3). Eosinophilic granuloma is a nonmalignant proliferative disease which primarily affects children and is characterized by osteolytic lesions associated with a granulomatous proliferation of cells including polymorphonuclears, lymphocytes, monocytes and a predominant cell type with special characteristics called histiocytosis X cells. Histiocytosis X cells show striking similarities to Langerhans cells of the skin (4). Both cell types adhere to plastic surfaces, show typical intracytoplasmic granules in electron microscopy studies, and bear Dr and T6 antigens, as shown by immunocytochemistry studies using monoclonal antibodies (5–7). Preparations rich in skin Langerhans cells have been shown to secrete interleukin 1 (IL-1) in culture (8). Apart from its immunological effects (9), IL-1 (or closely related molecules) is able to exert a series of functions associated with inflammation (10) and to stimulate bone resorption in vitro (11). Prostaglandins of the E series (PGE) have also been implicated in the lysis of bone tissue (12). IL-1 and PGE_2, as bone resorpting agents, may thus have a role in the osteolysis observed in vivo when secretory cells infiltrate bones. In the case of eosinophilic granuloma, one is tempted to postulate a role for the predominant cell type found in the lesion (the histiocytosis X cells), in the osteolysis phenomenon. The aim of the present study was thus to show that histiocytosis X cells, surgically collected from patients with bone granuloma and purified by fluorescein-conjugated monoclonal anti-T6 antibody and the use of a fluorescence activated cell sorter (FACS), are indeed able to secrete IL-1 and PGE_2 in vitro.

Received for publication 2 April 1985 and in revised form 9 October 1985.

1. *Abbreviations used in this paper:* CM, control medium; FACS, fluorescence-activated cell sorter; IFN γ, interferon γ; IL-1, interleukin 1; IL-2, interleukin 2; MCF, mononuclear cell factor; OAF, osteoclast activating factor; PGE, prostaglandin E; PGE_2, prostaglandin E_2; PHA, phytohemagglutinin P; ^3HT, tritiated thymidine.

J. Clin. Invest.

0021-9738/86/01/0326/04 $1.00
Volume 77, January 1986, 326–329

Methods

Specimens. Biopsy specimens from two cases of eosinophilic granuloma taken from skull lesions were used in this study. The two male patients, J.A. (4-yr-old) and T.P. (10-yr-old), showed clinically, radiologically, and histologically typical eosinophilic granuloma of the skull. The specimens were divided into three parts. The first was immediately placed in buffered formaldehyde (15%) for histopathological observation. The second was fixed in buffered glutaraldehyde for electron microscopic examination. The third was used for cultures.

Cell suspensions. Cell suspensions obtained from biopsy specimens were filtered through a sterile gauze, and cell viability was evaluated by trypan blue exclusion test. Thereafter, cells were either directly placed in culture (see below) or frozen and stored in liquid nitrogen until use.

Purification of histiocytosis X cells. Frozen ampoules containing eosinophilic granuloma cell suspension were thawed. After two washes, the cells were mixed with fluorescein-conjugated OKT6 monoclonal antibody (Ortho Pharmaceutical Corp., Raritan, NJ) at 1/10 dilution in phosphate-balanced salt solution (PBS), and placed in an ice bath for 30 min. After two washes in Hanks' solution deprived of Ca^{++} and Mg^{++} (Eurobio, Paris, France) and supplementd with 5 mM EDTA, cells were resuspended in the same medium at the concentration of 2×10^6 cells/ml. The cell suspensions were analyzed on a cytofluorograph (model 50L; Ortho Instruments, Westwood, MA) equipped with an Argon-ion laser emitting at 488 nm (40 mW). Sorting was performed by using two parameters: green fluorescence (detected through a narrow band interference filter 518–545 nm) and forward angle scatter (detected through a neutral density filter).

Cell culture conditions. Whole eosinophilic granuloma cell suspensions were placed into 60-mm plastic Petri dishes (Nunc, Roskilde, Denmark) at 5×10^6 cells/ml for 2 h at 37°C in RPMI 1640 (Gibco, Paisley, Scotland) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 5 mM Hepes and 5% of a unique pool of human AB serum (control medium, CM). Under these conditions most eosinophilic granuloma cells readily adhered to plastic. Nonadherent cells were removed by gentle washes with Hanks' solution. The remaining adherent cells were detached by gentle pipetting after 15 min incubation

with cold PBS containing 0.1% EDTA, and thoroughly washed. Both adherent eosinophilic granuloma cells and FACS-purified HX cells were suspended in fresh CM, seeded in flat-bottomed microplates (Nunc) at 2×10^5 cells/ml, 0.2 ml/well, and cultured for various periods of time. When indicated, purified recombinant human interferon (rIFN γ, a kind gift from Dr. W. Fiers, Gent, Belgium) produced in chinese hamster ovary cells was added to the cultures at a concentration of 1,000 IU/ml. Lipopolysaccharide (*Escherchia coli* 026: B6, Sigma Chemical Co., St. Louis, MO) was used as inducer of either IL-1 or PGE_2 at a concentration of 20 μg/ml. Supernatants were harvested after centrifugation at 1,000 *g* for 10 min and stored at −20°C.

IL-1, interleukin 2 (IL-2), and IFN assays. IL-1 activity was tested as previously described (13). Briefly, serial $\log_2$ dilutions of supernatants were performed in flat-bottomed microplates. In each well, 100 μl of a suspension of thymocytes from 4–8-wk-old C3H/HeJ mice (Centre d'élevage d'animaux de laboratoire, Orléans, France) containing 1.5×10^6 cells was added. Phytohemagglutinin P (PHA, Difco Laboratories, Inc., Detroit, MI) was added at a final concentration of 1/2,000. Cell cultures were incubated for 72 h at 37°C in a 5% Co_2 atmosphere. 18 h before harvest, cultures were pulsed with 1 μCi/ml of tritiated thymidine (^{3}HT, CEA, Saclay, France, specific activity 5 Ci/mmol). IL-2 activity was measured by using a standard microassay based on the IL-2–dependent exponential proliferation of a murine T cell line as previously described (14). IFN assay was performed using fibroblast cultures and vesicular stomatitis virus, as previously described (15).

PGE_2 *assay.* PGE_2 in supernatants was measured by radioimmunoassay, as previously described (14).

Table I. IL-1 Activity in Supernatants of Adherent Granuloma Cell Cultures from a Child with an Eosinophilic Granuloma*

Incubation conditions	Days of preincubation‡	Lipopoly-saccharide	IL-1 activity at supernatant dilutions of:§ 1/2	1/4	1/8	1/16
		20 μg/ml	*cpm*	*cpm*	*cpm*	*cpm*
Medium	0	−	4,313	4,787	3,836	3,167
		+	12,988	8,979	6,476	5,024
Medium	7	−	1,769	1,096	1,049	753
		+	8,177	3,152	2,330	1,934
rIFN γ	7	−	987	804	958	1,583
		+	30,145	19,032	14,274	11,294

* Il-1 activity (defined biologically as lymphocyte-activating factor) was measured by the mitogenicity of supernatants on murine thymocyte cultures and expressed in counts per minute of ^{3}HT incorporation. Lipopolysaccharide (ether extract from *E. coli*) was used to trigger IL-1 secretion, and rIFN γ was used to activate the cells and enhance their secretory potential.

‡ Cell cultures were stimulated with lipopolysaccharide either immediately after seeding (day 0) or after 7 d incubation in medium with or without rIFN γ (1,000 IU/ml).

§ Supernatants were collected 3 d after lipopolysaccharide induction. The background incorporation of ^{3}HT by thymocytes stimulated with PHA or PHA plus lipopolysaccharide were 992 and 650 cpm, respectively. Thymocytes in culture medium alone incorporated 430 cpm.

Results

Morphologic features and T6 antigen expression. In hematoxilin-eosin–stained sections, the two biopsy specimens investigated showed variable numbers of atypical mononuclear cells, with irregularly folded and grooved nuclei with fine chromatin. Few fibroblasts, red blood cells, neutrophils, eosinophils, lymphocytes, and plasma cells were also observed. Electron microscopy revealed pentalaminar cross-striated cytoplasmic structures consistent with Langerhans granules in the majority of cells which composed the lesion. These cells are known to be $T6^+$. The analysis of the histogram of green fluorescence intensity, obtained on the cytofluorograph, indicated that 75% of the cells were $T6^+$ and 25% were $T6^-$. These results corroborate fluorescence microscopic estimations on aliquots of the same cell suspensions.

Whole adherent eosinophilic granuloma cells secrete IL-1 in culture. As seen in Table I, the supernatant of 3-d cultures of adherent cells from eosinophilic granuloma biopsies was found to contain a clearly detectable IL-1 activity. Thus, spontaneous IL-1 secretion was observed in unstimulated cells under our culture conditions. IL-1 activity was much higher in cultures stimulated with lipopolysaccharide. Table I also shows that the lipopolysaccharide-induced IL-1 secretion was clearly enhanced when the cell cultures were incubated with rIFN γ for 7 d before lipopolysaccharide stimulation. We used 1,000 IU/ml IFN γ, since this concentration was previously found to result in maximal enhancement of IL-1 secretory function in blood monocyte cultures (14). rIFN γ pretreatment of non–lipopolysaccharide-stimulated cells did not induce IL-1 secretion, or resulted in a slight (nonsignificant) decrease of IL-1 activity (as seen in the experiment shown in Table I). No IL-2 activity could be found in the supernatants.

FACS-purified, $T6^+$ eosinophilic granuloma cells secrete IL-1. $T6^+$ cells were purified from whole eosinophilic granuloma cell preparations (from patient TP) with a FACS, under clear discriminative conditions, as seen in Fig. 1. Such highly purified preparations were found to spontaneously secrete IL-1. Two experiments were performed and provided comparable results, one experiment being shown in Fig. 2. Stimulation with lipopolysaccharide resulted in enhanced IL-1 activity in supernatants. Incubation of cell cultures in medium containing rIFN resulted in a clear increase of lipopolysaccharide-induced IL-1 secretion. In no instance was IL-2 activity detectable in such supernatants, whatever culture conditions used.

PGE_2, IL-1, IL-2, and IFN activity in supernatants from unseparated eosinophilic granuloma cells or purified histiocytosis X cell cultures. As shown in Table II, PGE_2 was spontaneously secreted by unstimulated cells from either unseparated adherent eosinophilic granuloma cells or FACS-purified $T6^+$ cells. The levels of PGE_2 were low, but significantly superior to the background titer found in culture medium. PGE_2 secretion was not enhanced in lipopolysaccharide-stimulated cultures. It should be noted that no IL-2 or IFN activity could be detected in any supernatant.

Discussion

The results shown in the present report indicate that cell cultures originating from surgically collected skull lesions of two patients

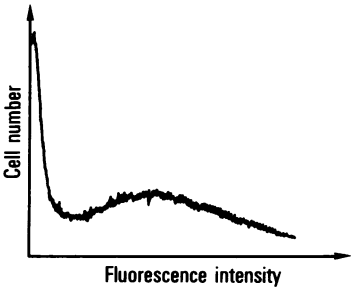

Figure 1. Pattern of cell sorting on a FACS, using OKT6-labeled eosinophilic granuloma cells. Details as described in Methods.

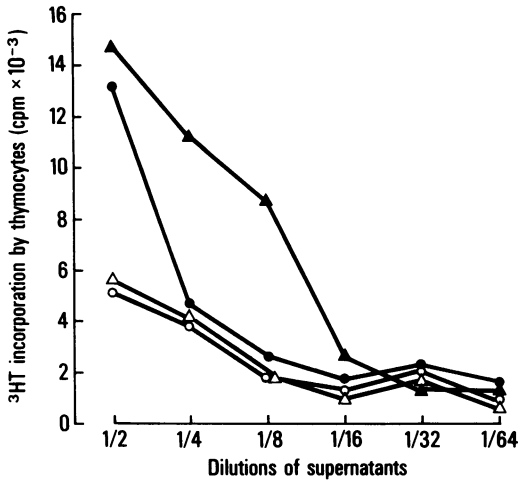

Figure 2. Adherent bone granuloma's cells from one patient (T.P.) were stained with fluoresceined OKT6 antibody and processed by a FACS. $T6^+$ cells were cultured at concentrations of 2×10^5/ml, and either stimulated or not by lipopolysaccharide for 3 d. At day 3, supernatants of the cultures were harvested and tested for IL-1 activity. Thymocytes stimulated by PHA or PHA plus lipopolysaccharide incorporated 600 and 1,773 cpm of 3HT, respectively. Thymocytes stimulated with PHA or PHA plus lipopolysaccharide in the presence of rIFN γ (1,000 IU/ml) incorporated 732 and 1,635 cpm of ^{3}HT, respectively. ▲, IFN γ plus lipopolysaccharide. ●, lipopolysaccharide. △, IFN γ. ○, control medium.

with eosinophilic granuloma produce IL-1 and PGE_2. The IL-1 nature of the mitogenic activity found in culture supernatants was ascertained by its effects on murine thymocyte proliferation, contrasting with its lack of mitogenic effect on an IL-2–dependent murine T cell clone. IL-1 secretion by eosinophilic granuloma cells was intense, comparable to the secretion observed in monocyte cultures optimally stimulated under identical culture conditions (14). The cellular nature of eosinophilic granuloma is not homogeneous. Although the main cell type found in the lesions is the characteristic histiocytosis X cell, the presence of a minority of common macrophage is well documented, and was indeed detected in unseparated cells from the two biopsy specimens investigated in the present work. Since macrophages are known to be able to secrete IL-1 and PGE_2 (16), the possibility existed that macrophages, rather than (or in addition to) histiocytosis X cells, produced the two mediators detected in our cultures. We have thus taken advantage of the strong T6 positivity of histiocytosis X cells (and of the complete negativity of tissue macrophages for this membrane antigen) to purify histiocytosis X cells from preparations of eosinophilic granuloma lesions under highly discriminative conditions. Highly purified T6-positive cells were found to produce both IL-1 and PGE_2 in culture. This finding documents a new functional property of histiocytosis X cells, and provides another similarity between histiocytosis X cells and skin Langerhans cells. Indeed, the latter cell type has been shown to produce IL-1 in vitro (7). This is a further argument in favor of the concept that histiocytosis X cells and skin Langerhans cells are of the same lineage (4).

Table II. PGE_2 Content, IL-1 and IL-2 Activity of Supernatants from Whole Granuloma or T6+-sorted Cell Population

Cell preparation*	Lipopolysaccharide (20 g/ml)	IL-1‡§	PGE_2‖	IL-2	IFN
		cpm	*pg/ml*		*IU/ml*
Unseparated	–	2,066	486	Undetectable	<3
	+	6,380	276	Undetectable	<3
T6+ sorted	–	5,100	400	Undetectable	<3
	+	13,200	300	Undetectable	<3

* Patient T.P.: 2×10^5 cells/well in a final volume of 200 liters. Stimulation at day zero.

‡ Background titers of PGE_2 and IL-1 are seen in nonlipopolysaccharide-stimulated cultures.

§ Supernatant tested at the concentration of one-half. The background incorporation of ^{3}HT by thymocytes stimulated with PHA or PHA plus lipopolysaccharide were, respectively, 325 and 1,235 cpm in the experience with unseparated cells, and 600 and 1,173 cpm in that with T6+-purified cells. Thymocytes in culture medium incorporated 312 cpm.

‖ PGE_2 content of culture medium used in this experiment was 66 pg/ml.

The secretion of IL-1 by eosinophils granuloma cells was found to occur spontaneously under our culture conditions. This was the case with cultured adherent cells, and even with unmanipulated biopsies simply left as tissue explants in culture medium (data not shown). Our culture conditions were chosen to avoid any known IL-1 triggering, i.e., use of high quality medium, and human rather than bovine serum. Under similar conditions, and indeed in parallel experiments during the completion of this work, human blood monocytes do not spontaneously secrete IL-1, as discussed previously (14). Whether this spontaneous in vitro production truly reflects a permanent production of IL-1 by histiocytosis X cell in the bone lesions remains a matter of speculation. $T6^+$ cells, representing the large majority (75–90%) of our cell preparations as analyzed by the cytofluorograph and showing an intense IL-1 secretory potential in vitro, are clearly excellent candidates as primary IL-1 secretory cell type within bone lesions. The question arises of whether $T6^-$ cells also produce IL-1. The few ($<10^4$) $T6^-$ cells left viable after sorting were not found to produce IL-1 (data not shown). This clearly cannot exclude, however, the participation of T6 negative cells in local Il-1 production. The finding of a spontaneous IL-1 secretion by cells in culture does not necessarily reflect the maximal IL-1 secretory potential of the culture. Indeed, our observations in human peripheral blood monocytes indicate that lipopolysaccharide-induced IL-1 secretion is rapidly lost in culture and that IFN γ can reconstitute and enhance the secretory potential of aged monocyte cultures (15). We have used this approach in the present system, and found that lipopolysaccharide induces high levels of IL-1 production in culture of either unseparated or FACS-sorted $T6^+$ eosinophilic granuloma cells. Furthermore, treatment with IFN γ before stimulation with lipopolysaccharide revealed an intense secretory potential of histiocytosis X cells in culture. The finding that IFN γ, a T cell lymphokine, increases the IL-1 secretory function of histiocytosis X cells is compatible with the hypothesis that T lymphocytes may participate in the regulation of IL-1 secretion within eosinophilic granuloma lesions, thus perpetuating the local secretion of this inflammatory monokine.

In contrast with what we have shown with IL-1 secretion, the production of PGE_2 was found to be modest quantitatively

and not enhanced in vitro by treatment of eosinophilic granuloma cells with lipopolysaccharide. This is in agreement with a previous report (17) showing that stimulation does not increase the secretion of PGE and thromboxane in cultures of adherent cells originating from the lungs or the lymph node of a patient who died of a disseminated form of histiocytosis X.

The question arises of whether the secretion of IL-1 and PGE_2 by eosinophilic granuloma cells has a role in the bone tissue damage resulting in the characteristic osteolytic lesions of the disease. Indeed, histiocytosis X, as a systemic disease, appears to be one of the most tissue-destructive syndromes, able to induce multiple and grossly apparent lytic lesions involving many organs, including the basal layer or the epidermis, the lung, and the bones. Since this lytic activity cannot be connected with a neoplastic nature of the disease, one can reasonably assume that it may be related to the local release of some tissue-lytic factor(s). We have previously reported that eosinophilic granuloma cells are able to exert a measurable collagenolytic activity in vitro (18). Purified IL-1 preparations have been shown to stimulate bone resorption in vitro (11). IL-1 preparations described in reference 11 show mitogenic activities in thymocyte proliferation assays similar to the IL-1 activity found in our histiocytosis X cell supernatants. The effect of IL-1 in bone resorption is apparently direct, and is not inhibited by indomethacin. In this respect, IL-1 behaves differently than the elusive mediator operationally named osteoclast activating factor (OAF), which is claimed to be PGE-dependent for both its production and its effect on bone resorption (12, 19). On the other hand, mononuclear cell factor (MCF), a mediator considered to be very similar or identical to IL-1, has been shown to induce PGE and collagenase production by synovial cells in culture (20, 21). Finally, monocyte supernatants have been shown to stimulate the production of proteinase and PGE by chondrocytes and bone cells (22), and indomethacin inhibits PGE synthesis and calcium release induced by OAF-rich lymphokine preparations in explanted fetal rat bone (11). Our present findings thus open up the possibility that histiocytosis X cells induce bone resorption in eosinophilic granuloma through their ability to secrete locally IL-1 and PGE. The intense secretion of IL-1 that we have detected in both eosinophilic granuloma explants and purified histiocytosis X cells in culture is indeed likely to have a major role in local osteolysis, either directly or through the induction of PGE by neighboring cells in the lesion. The demonstration of a secretory function of histiocytosis X cells may thus be relevant to our understanding of the pathogenesis of histiocytosis X tissue lesions.

Acknowledgments

The expert technical assistance of Annie Munier, Anne Claude Waché, and Hélène Mouly is gratefully acknowledged. Mrs. Martine Grimal typed the manuscript. We are indebted to V. Blanca Paloma for critical help in cell-sorting experiments.

This work was supported in part by grants from University Paris VII.

References

1. Lichetenstein, L. 1953. Histiocytosis X: integration of eosinophilic granuloma of bone, Letterer-Siwe disease and Hand-Schüller-Christian disease as related manifestations of a single nosologic entity. *Arch. Pathol.* 56:84–102.

2. Nezelof, C. 1979. Histiocytosis X: a histological and histogenetic study. *In* Pediatric Pathology. H. S. Rosemberg and R. P. Bolande, editors. Masson, Paris. 5:153–178.

3. Farber, S. 1941. The nature of solitary or eosinophilic granuloma of bone. *Am. J. Pathol.* 17:625–630.

4. Nezelof, C., F. Basset, and M. F. Rousseau. 1973. Histiocytosis X: histogenetic arguments for a Langerhans cell origin. *Biomedicine (Paris).* 18:365–371.

5. Murphy, G. F., A. K. Bhan, S. Sato, T. J. Harrist, and M. C. Mihm. 1981. Characterisation of Langerhans cells by the use of monoclonal antibodies. *Lab. Invest.* 45:465–468.

6. Chollet, S., P. Dournovo, M. S. Richard, P. Soler, and F. Basset. 1982. Reactivity of histiocytosis X cells with monoclona anti-T6 antibody. *N. Engl. J. Med.* 307:685.

7. Rousseau-Merck, M. F., S. Barbey, F. Jaubert, M. A. Bach, L. Chatenoud, and C. Nezelof. 1983. Reactivity of histiocytosis X cells with monoclonal antibodies. *Pathol. Res. Pract.* 177:8–12.

8. Sander, D. N., C. Dinarello, and V. B. Morhenn. 1984. Langerhans cell production of interleukin 1. *J. Invest. Dermatol.* 82:605–607.

9. Oppenheim, J., and I. Gery. 1982. Interleukin 1 is more than an interleukin. *Immunol. Today.* 3:113–119.

10. Dinarello, C. 1984. Interleukin 1 and the pathogenesis of the acute-phase response. *N. Engl. J. Med.* 311:1413–1418.

11. Gorven, M., D. D. Wood, E. J. Ihvie, M. K. B. McQuire, and R. G. G. Russell. 1983. An interleukin 1-like factor stimulates bone resorption in vitro. *Nature (Lond.).* 306:378–380.

12. Bockman, R. S., and M. A. Repo. 1981. Lymphokine-mediated bone resorption requires endogenous prostaglandin synthesis. *J. Exp. Med.* 154:529–534.

13. Arenzana-Seisdedos, F., and J. L. Virelizier. 1983. Interferons as macrophage-activating factors. II. Enhanced secretion of interleukin 1 by lipopolysaccharide-stimulated human monocytes. *Eur. J. Immunol.* 13:437–440.

14. Wakasugi, N., J. L. Virelizier, F. Arenzana-Seisdedos, B. Rothhut, J. M. Mencia-Huerta, F. Russo-Marie, and W. Fiers. 1985. Defective IFN production in the human neonate. II. Role of increased sensitivity to the suppressive effects of prostaglandin *Eur. J. Immunol.* 134:172–176.

15. Arenzana-Seisdedos, F., J. L. Virelizier, and W. Fiers. 1985. Interferons as macrophage-activating factors. III. Preferential effects of interferon on the interleukin 1 secretory potential of fresh or aged human monocytes. *J. Immunol.* 134:2444–2448.

16. Nathan, C. F., H. W. Murray, and Z. A. Cohn. 1980. The macrophage as an effector cell. *N. Engl. J. Med.* 303:622–626.

17. Gonzalez-Crussi, F., W. Hsueh, and M. D. Wiederhold. 1981. Prostaglandin in histiocytosis X. P. G. synthesis by histiocytosis X cells. *Am. J. Clin. Pathol.* 75:243–253.

18. Rousseau-Merck, M. F., S. Barbey, H. Mouly, S. Bazin, and C. Nezelof. 1979. Collagenolytic activity of eosinophilic granuloma in vitro. *Experientia (Basel).* 35:1226–1227.

19. Yoneda, T., and G. R. Mundy. 1979. Monocytes regulate osteoclast-activating factor production by releasing prostaglandins. *J. Exp. Med.* 150:338–350.

20. Dayer, J. M., J. Breard, L. Chess, and S. M. Krane. 1979. Participation of monocyte-macrophages and lymphocytes in the production of a factor that stimulates collagenase and prostaglandin release by rheumatoid synovial cells. *J. Clin Invest.* 64:1386–1392.

21. Mizel, S. B., J. M. Dayer, S. M. Krane, and S. E. Mergenhagen. 1981. Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activation factor (interleukin 1). *Proc. Natl. Acad. Sci. USA.* 78:2474–2477.

22. McQuire, M. K. B., J. E. Meats, N. M. Ebsworth, G. Murphy, and G. G. Russel. 1982. Messenger function of prostaglandins in cell to cell interactions and control of proteinase activity in the rheumatoid joint. *Int. J. Immunopharmacol.* 4:91–102.