Immunology 1982 **47** 531

Altered density, metabolism and surface receptors of eosinophils in eosinophilia

I. WINQVIST, T. OLOFSSON, I. OLSSON, ANN-MAJ PERSSON & T. HALLBERG *Department of Internal Medicine and Department of Bacteriology, University of Lund, Sweden*

Accepted for publication 25 May 1982

Summary. A comparison was made between eosinophils from normal persons and patients with eosinophilia. Highly purified eosinophils were obtained by centrifugation in a Percoll density gradient. Studies were carried out on density distribution, oxygen consumption upon adherence to serum-coated Sephadex and expression of cell surface receptors for IgG and complement. In eosinophil leukaemia the density of eosinophils was abnormally low. Abnormal light density fractions of blood eosinophils were also detected in the hypereosinophilic syndrome (HES). Light density eosinophils of HES showed morphological signs of degranulation consonant with the finding of a low content of granular eosinophil cationic protein (ECP) suggesting degranulation in the circulation or abnormal granule formation in the marrow. In addition, such cells exhibited a higher oxygen consumption than eosinophils with normal density upon adherence to serum-coated Sephadex. Low density eosinophils showed a greater number of cells with Fc-IgG and complement receptors than high density cells. Likewise exudate eosinophils displayed an abnormally low density with higher than normal oxygen consumption indicating that eosinophils may be activated in the tissues. In one patient with HES, a febrile episode resulted in a disappearance of eosinophils with a normal density while abnormal low density eosinophils increased. Our findings suggest that eosinophils from some patients with eosinophilia may be 'activated' in the circulation.

Correspondence: Dr I. Winqvist, Department of Internal Medicine, Lund Hospital, S-221 85 Lund, Sweden.

0019-2805/82/1100-0531$02.00

INTRODUCTION

Eosinophilia is typically associated with allergic and parasitic disease but it can also be a feature of other disorders such as immune complex diseases. The function of the eosinophil is a matter of controversy. One concept suggests a modulatory role in the anaphylactic reaction (Goetzl, Weller & Valone, 1979) and another a role in killing of parasites (Kay, 1979). Eosinophil granules contain unique proteins such as the major basic protein (MBP; Gleich, Loegering, Kueppers, Bajaj & Mann, 1974; Gleich, Loegering, Mann & Maldonado, 1976; Gleich, Loegering & Maldonado, 1973) and the eosinophil cationic protein (ECP; Olsson & Venge, 1974; Olsson, Venge, Spitznagel & Lehrer, 1977). These proteins were toxic to schistosomules of *Schistosoma mansoni* (Butterworth, Wassom, Gleich, Loegering & David, 1979; McLaren, McKean, Olsson, Venge & Kay) and to a variety of mammalian cells (Gleich, Frigas, Loegering, Wassom & Steinmüller, 1979; Frigas, Loegering & Gleich, 1981). Extracellular release of such agents with damage to tissues may therefore be suggested as responsible for cardiovascular complications of the hypereosinophilic syndrome (Spry, 1980). Eosinophil production of oxygen products including superoxide anion, hydroxyl radical, and hydrogen peroxide might also lead to tissue damage. In certain states of high

eosinophilia, eosinophils could be activated both to release granular cationic proteins and produce active oxygen products. Morphological abnormalities including vacuolated and degranulated eosinophils as well as increased rosette formation with antibody-coated erythrocytes (Tai & Spry, 1976) support this view. Furthermore eosinophils from patients with eosinophilia appeared activated when compared with normal eosinophils by surface charge, activation of acid phosphatase, membrane hexose transport, and hexose monophosphate shunt activities (Bass, Grover, Lewis, Szedja, de Chatelet & McCall, 1980).

The present work was undertaken to compare eosinophils from normal persons with those from patients with eosinophilia. We performed studies on density distribution, oxygen consumption upon adherence to serum-coated Sephadex (Metcalf, Gadek, Raphael, Frank, Kaplan & Kaliner, 1977) and expression of cell surface receptors for IgG and complement. Our results indicate that 'activated' eosinophils circulate in certain hypereosinophilic states.

MATERIALS AND METHODS

The patient material is presented in Table 1. No treatment had been given to these patients.

Isolation of eosinophils by centrifugation in a Percoll density gradient

Heparinized blood was collected from healthy volunteers (eosinophils $< 400/\mu l$) and from volunteers with eosinophilia (Table 1). Five parts blood were mixed with one part 6% Dextran (Dextran 250, Pharmacia Fine Chemicals, Uppsala, Sweden) in 0·15 M NaCl and left at room temperature for 45 min to allow the red cells to sediment. The dextran-plasma was collected and centrifuged at 450 *g* for 5 min. The cells were washed twice in Hanks's balanced salt solution (HBSS; Flow Laboratories).

A density gradient of Percoll (polyvinylpyrrolidone-coated silica gel, Pharmacia Fine Chemicals, Uppsala, Sweden) was made using a modification of Gärtner (1980). Percoll and HBSS were mixed to obtain solutions of different densities at pH 7·4. The osmolarity was adjusted with either HBSS or water to 275 mosm. Gradients were formed using a peristaltic pump (Minipuls II Gilson, France) and consisted of Percoll solutions with the following densities (g/ml): 1·100 (1 ml), 1·095 (3 ml), 1·090 (3 ml) and 1·085 (3 ml) layered on top of each other in 17 × 100 mm polypropylene tubes (Falcon Plastics, Los Angeles, Calif.). After washing, the cells were suspended in Percoll (1·075 g/ml) and adjusted to 25×10^6/ml. Two millilitre of the cell suspension were layered on each gradient followed by centrifugation in an angle rotor (angle 45°) at 1600 *g* for 20 min at room temperature. One millilitre fractions were collected from the bottom of the tubes using a peristaltic pump. The density of each fraction was measured and the cells were washed in HBSS and counted. Cytocentrifuge smears were prepared for differential counts.

Assay for eosinophil cationic protein

Isolated granulocytes were extracted with 0·3% cetyltrimetyl ammonium bromide (CTAB, BDH, Poole) in 0·01 M sodium phosphate buffer pH 7·0. One millilitre

Table 1.

	Patients	Age	Sex	WBC × 10^9/litre	Eosinophils (%)
A.	Hypereosinophilic syndrome	48	M	20.3	45·3
	Hypereosinophilic syndrome	36	F	28·9	57·4
	Eosinophil leukaemia	25	M	11·5	47·0
	Sideroblastic anaemia + eosinophilia	69	M	11·9	61·5
B.	Polymyalgia rheumatica	83	M	6·6	13·5
	Rheumatoid arthritis	63	F	12·6	39·7
C.	Filariasis	34	M	70·0	85·5
	Anchylostomiasis	29	M	9·1	36·0
D.	Hodgkin's lymphoma	57	M	15·4	57·7
	Primary hepatocellular carcinoma	54	F	40·8	9·0
	Eosinophil gastroenteritis	64	F	6·5	41·0

of this solution was added per 10^7 cells and extraction was carried out at 0° with frequent mixing using a pasteur pipette. ECP was quantified with the single radial immunodiffusion method of Mancini, Carbonara & Heremans (1965) using an antiserum against ECP (Venge, Roxin & Olsson, 1977a). Purified ECP (Olsson *et al.*, 1977) was used as reference standard.

Oxygen consumption

Oxygen consumption was stimulated with serum-treated Sephadex beads (Pharmacia, Uppsala, Sweden). The Sephadex beads were suspended in HBSS ($0{\cdot}3 \times 10^6$/ml). This suspension (0·8 ml) was incubated with 0·7 ml normal human serum (stored at −80°) at 37° for 10 min and mixed with 1 ml cell suspension to a final concentration of 4×10^6 cells/ml (purity 82%–97%). Thereafter oxygen consumption was measured with a Clark electrode mounted in a 2·24 ml glass-stoppered incubation chamber equipped with magnetic stirring and heating to 37° (Eschweiler & Co, Kiel, Germany). A paper recorder was connected for continuous recording of the oxygen tension. The electrode was calibrated with pure N_2 and 12·25% oxygen in N_2. Oxygen consumption, expressed as μl $O_2/10^7$ granulocytes/min, was calculated according to the following formula:

$$\text{ml } O_2 = pO_2 \times \frac{\alpha}{760} \times V$$

where α is the absorption coefficient (0·02350) and V is the volume of the reaction chamber (2·24 ml).

Cell surface receptors

Fc receptors for IgG were determined by a rosette assay using ox erythrocytes coated with rabbit antibodies (Hallberg, Gurner & Coombs, 1973). Complement receptors were determined by a rosette assay using sheep erythrocytes coated with mouse complement. For this test sheep erythrocytes coated with a small dose of rabbit IgM antibodies were incubated with an optimal amount of fresh mouse serum (strain A/SN) for 15 min at 37°. After repeated washes the cells were made up to 1% in Dulbecco's saline with 0·2% bovine serum albumin. One hundred microlitres of cells (2×10^6/ml) and 100 μl of indicator red cells were mixed in a 3 ml plastic tube and rotated for 1 hr at 37°. Aliquots of the cell suspensions were then mixed with toluidine blue dye and counted in a microscope as for the Fc rosette assay. Each test contained controls with untreated sheep erythrocytes as well as sheep erythrocytes coated with rabbit antibodies but lacking mouse complement. These controls were consistently negative.

RESULTS

Density distribution of eosinophils and relationship to content of ECP

Figure 1 shows the density distribution in Percoll of eosinophils from healthy individuals. The recovery of eosinophils was 42%–62%. The peak density is 1·088 g/ml. Neutrophils were found to have a lower density with a peak at 1·081 g/ml.

An identical distribution was found in the density gradient of eosinophils and immunochemically determined ECP (data not shown) both in healthy individuals and patients with eosinophilia, but not in eosinophil leukaemia. These findings would be expected if ECP is a unique constituent of eosinophils. The relationship between density of eosinophils and content of ECP was also analysed (Fig. 2).

Eosinophils with a low density had a lower content of ECP than those with a normal density. Therefore the ECP content is a parameter of cell density, which most likely is a function of the number and properties of eosinophil granules. However, in eosinophil leukaemia the content of ECP was very low and the

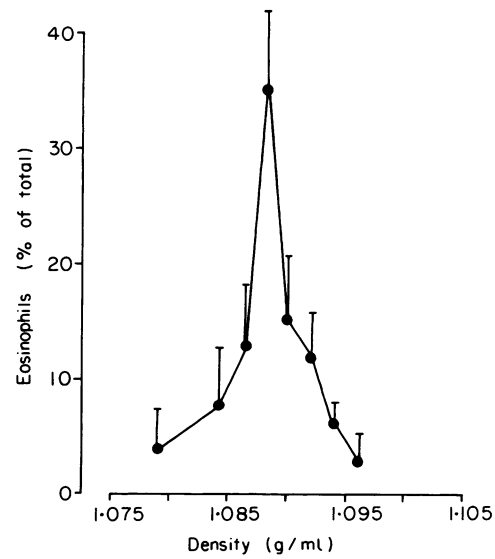

Figure 1. Density distribution of eosinophils from healthy individuals in a Percoll gradient ($n = 5$). Eosinophils peak at a density of 1·088 g/ml.

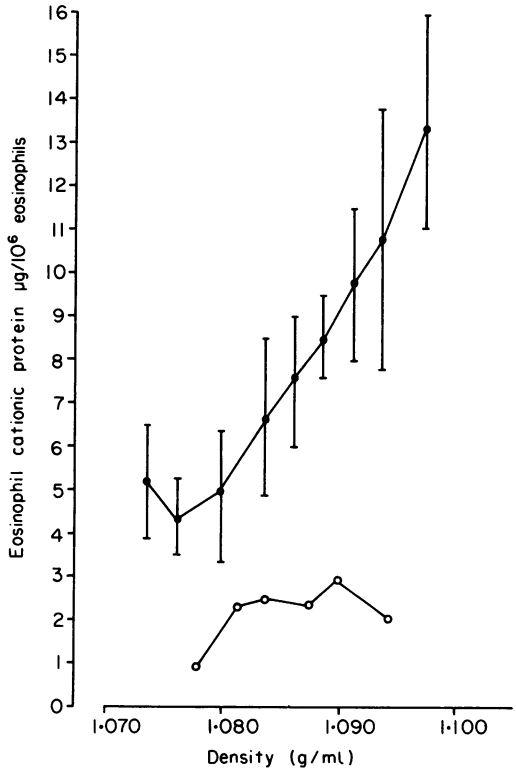

Figure 2. Relationship between density of eosinophils and content of ECP. Cells from nine patients with eosinophilia were centrifuged in Percoll. Eosinophils were recovered and assayed for ECP content (●——●). There is a decreasing content of ECP with decreasing cell density. Data on one patient with eosinophil leukaemia are presented separately (○——○) as they show abnormally low ECP content.

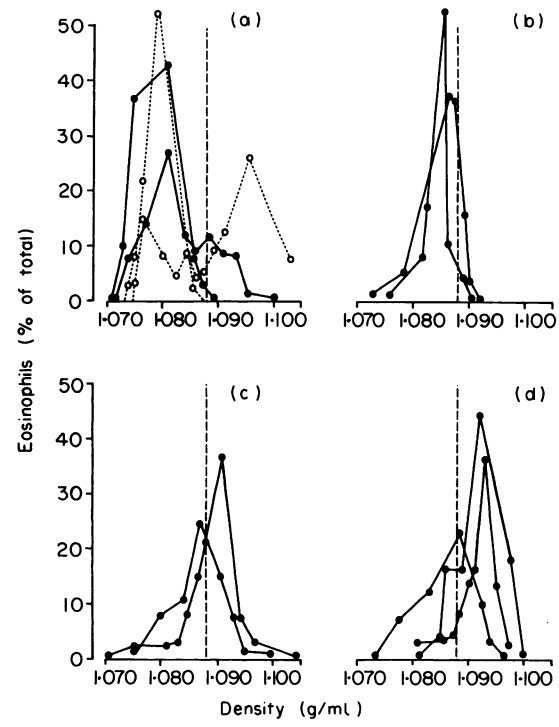

Figure 3. Density distribution of eosinophils from patients with eosinophilia. The broken vertical line indicates the peak density of normal eosinophils. Eosinophils from one patient with eosinophil leukaemia and one patient with sideroblastic anaemia, eosinophilia and abnormal karyotype (●——●) have abnormally low density while two patients with hypereosinophilic syndrome (○----○) show a more heterogenous pattern (a). In two patients with immune complex disease (rheumatoid arthritis and polymyalgia rheumatica) eosinophils show a normal density (b). Eosinophils from patients with parasite infections (c), and eosinophil gastroenteritis, primary hepatocellular carcinoma and Hodgkin's lymphoma (d) also exhibit a normal density.

relationship to cell density was less obvious compared to non-leukaemic eosinophilia.

Density distribution of eosinophils in eosinophilia

Figure 3 shows the density distribution of eosinophils from eleven patients with eosinophilia. Cells (50×10^6) were applied to each gradient. The number of eosinophils varied from $4{\cdot}5 \times 10^6$ to $42{\cdot}8 \times 10^6$. The recovery of eosinophils was 77·8% (56·0–98·0). In eosinophil leukaemia an abnormally low density was found. In hypereosinophilic syndrome (HES) eosinophils had either a low density or showed populations with both normal or low density. Eosinophils from patients with immune complex diseases (rheumatoid arthritis and polymyalgia) and eosinophilia displayed a normal density. Eosinophils from patients with parasite infections, eosinophil gastroenteritis, primary hepatocellular carcinoma and lymphoma also exhibited a normal density.

Eosinophil density, oxygen consumption and cell surface receptors in the hypereosinophilic syndrome

Figure 4 depicts studies of eosinophils from a patient with HES exhibiting both abnormally 'light' and 'heavy' eosinophils. The data of Fig. 4 are given in relative numbers. However, also when the data are given in absolute numbers a clear shift in density of the

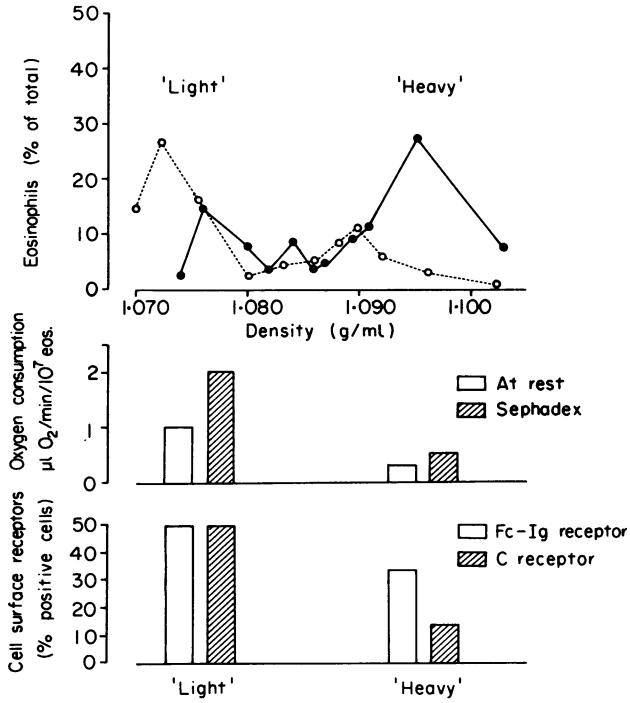

Figure 4. Studies of eosinophil density, oxygen consumption and cell surface receptors in a patient with hypereosinophilic syndrome (●——●); and during a febrile episode (o----o). Isolated 'light' and 'heavy' eosinophils were assayed for oxygen consumption at rest and after adherence to serum-treated Sephadex. Fc-IgG receptors and complement receptors were also determined.

eosinophils was seen during the febrile episode. Thus before the febrile episode $9{\cdot}6 \times 10^9$/l and $2{\cdot}7 \times 10^9$/l could be calculated to represent 'heavy' and 'light' eosinophils, respectively, per litre blood. During the febrile episode the numbers were $3{\cdot}74 \times 10^9$/l and $6{\cdot}59 \times 10^9$/l for 'heavy' and 'light' eosinophils, respectively. Striking differences were observed between light and heavy eosinophils (Fig. 4). Thus, oxygen consumption both at rest and upon adherence to serum-treated Sephadex was much higher for light than heavy eosinophils. Furthermore, 50% of light eosinophils showed Fc-IgG receptors and 50% showed complement receptors while heavy eosinophils displayed 34% and 14%, respectively.

Another HES patient with only abnormally light eosinophils (Fig. 3) showed 84% eosinophils with Fc-IgG receptors and 69% with complement receptors on their surface (data not shown).

In addition light eosinophils appeared degranulated as compared with heavy eosinophils (Fig. 5).

Comparisons between eosinophils from blood and pleural exudates

Comparative studies were done between blood and exudate eosinophils in one patient with eosinophilia ($1{\cdot}6 \times 10^9$ peripheral blood eosinophils/litre) and a pleural exudate with eosinophilia of unknown etiology (Fig. 6). Data for this patient are not included in Table 1. Blood eosinophils showed a normal density while pleural exudate eosinophils displayed an abnormally low density. Furthermore, pleural eosinophils showed a higher oxygen consumption than blood eosinophils (data not shown).

Oxygen consumption of eosinophils from patients with eosinophilia

These particular studies were carried out only with eosinophils from patients with eosinophilia because it was not possible to obtain enough eosinophils from normal persons. Figure 7 shows oxygen consumption

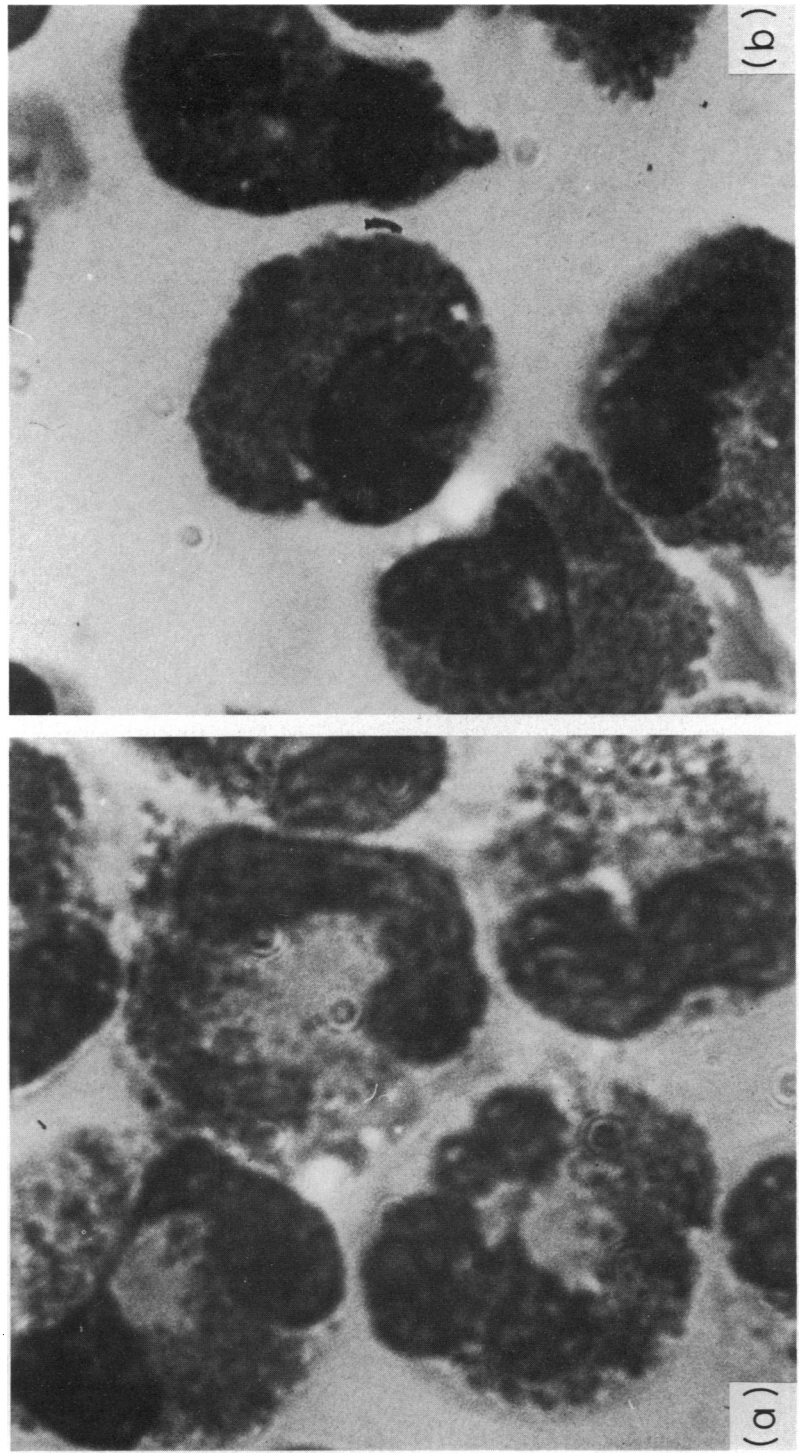

Figure 5. Eosinophils from the patient with hypereosinophilic syndrome described in Fig. 4 (a) 'Light', degranulated eosinophils. (b) 'Heavy' eosinophils with normal granulation.

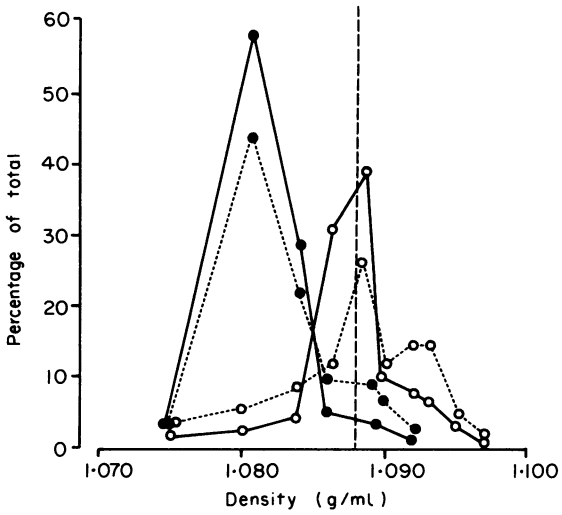

Figure 6. Comparisons of eosinophils isolated from blood and a pleural exudate in a patient with eosinophilia of unknown etiology. Density of blood eosinophils (O——O) and density of pleural eosinophils (●——●). Content of ECP of blood eosinophils (O----O) and pleural eosinophils (●----●).

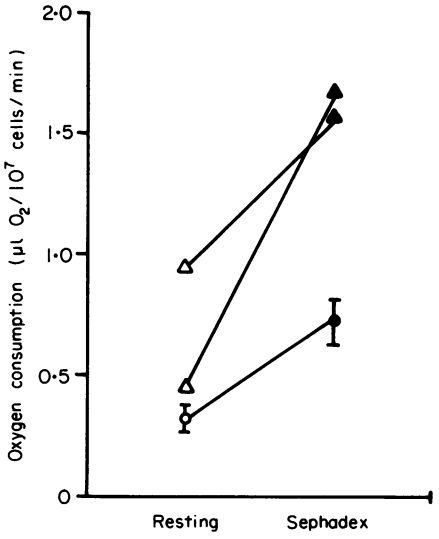

Figure 7. Oxygen consumption of eosinophils from patients with eosinophilia isolated by centrifugation in Percoll. 'Light' eosinophils from two patients with HES at rest (△) and during interaction with serum-treated Sephadex (▲). Eosinophils from six patients with eosinophilia (group b, c and d of Fig. 3) at rest (O) and during stimulation with serum-treated Sephadex (●).

for eosinophils at rest and during interaction with serum-treated Sephadex beads. The oxygen consumption usually increased two–three times upon contact with the opsonized beads. Light density eosinophils from two patients with HES showed a higher oxygen consumption upon contact with serum-treated Sephadex than eosinophils from other patients with eosinophilia or normal density eosinophils from patients with HES. Light eosinophils from one of these patients also exhibited an increase in oxygen consumption at rest (Figs 4 and 7).

Isolated neutrophils displayed a similar respiration as eosinophils at rest but only a minimal increase when added to the opsonized Sephadex beads (data not shown).

DISCUSSION

Our findings suggest that eosinophils from some patients with eosinophilia, especially those with the hypereosinophilic syndrome, may be 'activated' in the circulation. This is indicated by a decrease in cell density, increased expression of cell surface receptors, and increased oxygen consumption. It should, however, be emphasized that only a limited number of patients with this rare disorder could be investigated. Therefore, the present studies have to be repeated in a larger patient group before the clinical importance of the findings can be evaluated. Nevertheless, the present findings may offer explanations for the pathophysiology of HES.

The low density of eosinophils in HES can probably be explained by a partial degranulation in the circulation with release of granule contents. Alternatively, light density cells of HES may represent an abnormal (preleukaemic?) population which is produced in the marrow with a lower granule content than normal. But the correlation observed earlier between the number of morphologically appearing degranulated blood eosinophils and the serum level of the eosinophil cationic protein (ECP) determined by a radioimmunoassay (Venge *et al.*, 1977a) indicates that ECP is actually released in the circulation of patients with HES (Spry, 1980). Fluctuations of serum ECP have also been reported in patients with asthma (Venge, Zetterström, Dahl, Roxin & Olsson, 1977b; Dahl, Venge & Olsson, 1978) and other allergic diseases (Winqvist, Olsson, Werner & Stenstam, 1981). In the latter instances fluctuations of serum ECP may reflect release from circulating or tissue eosinophils although

changes in turnover rates also have to be considered. Furthermore, elevated concentrations of major basic protein of serum are present in some diseases associated with eosinophilia (Wassom, Loegering, Solley, Moore, Schooley, Fauci & Gleich, 1981). Thus, several observations suggest the possibility that eosinophils can be degranulated in certain disease states.

The abnormally low density of eosinophils from patients with eosinophil leukaemia indicating a low granule content or abnormal granule composition is probably a result of the malignant character of such cells rather than secondary degranulation. Thus, in contrast to findings in HES spontaneous and stimulated oxygen consumption were not elevated. Furthermore, the ECP content was extremely low in one patient with eosinophil leukaemia without a clear relationship to density of the cells, indicating abnormalities of granule composition. Otherwise we found a correlation between density distribution and ECP content of eosinophils from both healthy individuals and patients with eosinophilia strongly indicating that ECP is actually a unique eosinophil constituent (Olsson *et al.*, 1977). The normal density found for eosinophils of patients with parasite infections, eosinophil gastroenteritis, primary hepatocellular carcinoma and lymphoma suggest that eosinophil degranulation in the circulation is not a significant phenomenon in these disorders.

Our findings in HES of a mixture of eosinophils populations suggest activation by unknown mechanisms of some eosinophils. Light eosinophils of HES may represent a subpopulation of 'activated' eosinophils with a prolonged half-life in the circulation. Actually the blood half-life of Indium-labelled eosinophils was sometimes prolonged in hypereosinophilia (Spry, 1980). An activating process might explain for findings in such cells of increased expression of cell surface receptors, increased spontaneous oxygen consumption and an increased respiratory burst upon adherence to a complement-activated surface. It is at least unlikely that such changes reflect the eventual preleukaemic nature of eosinophils of HES even if leukaemic eosinophils were found to have an abnormal low density. Our finding of exudate eosinophils with lower density and higher spontaneous oxygen consumption than blood eosinophils suggests that eosinophils may be activated normally in the tissues to change their properties. In HES this process may occur prematurely in the circulation for unknown reasons and explain the presence of 'activated' eosinophils of low density. It is of interest that in one of our patients with HES, a febrile episode resulted in a profound decrease of 'heavy' eosinophils while 'light' density eosinophils actually increased. This process may represent activation in the circulation of eosinophils with a concomitant shift in cell density.

In a recent report eosinophils from patients with eosinophilia appeared activated when compared with normal eosinophils by surface charge, activation of acid phosphatase and hexose transport (Bass *et al.*, 1980). Interestingly, activation of oxidative metabolism was found separable from activation of cell charge or hexose transport. Both high spontaneous and/or stimulated respiration as found in the present study of some light density eosinophils as well as degranulation could result in cytotoxic damage to tissues in HES because of active oxygen products, and released ECP and MBP.

ACKNOWLEDGMENTS

This work was supported by the Swedish Cancer Society, Alfred Österlund Foundation, the Medical Faculty of Lund and the Swedish Medical Research Council (grant 5682).

REFERENCES

Bass D.A., Grover W.A., Lewis J.C., Szejda P., De Chatelet L.R. & McCall C.E. (1980) Comparison of human eosinophils from normals and patients with eosinophilia. *J. Clin. Invest.* **66,** 1265.

Butterworth A.E., Wassom D.L., Gleich G.J., Loegering D.A. & David J.R. (1979) Damage to schistosomula of *Schistosoma mansoni* induced by eosinophil major basic protein. *J. Immunol.* **122,** 221.

Dahl R., Venge P. & Olsson I. (1978) Variations of blood eosinophils and eosinophil cationic protein in serum in patients with bronchial asthma: studies during inhalation challenge test. *Allergy*, **33,** 211.

Frigas E., Loegering D.A. & Gleich G.J. (1981) Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab. Invest.* **42,** 35.

Gärtner I. (1980) Separation of human eosinophils in density gradients of polyvinyl pyrrolidone-coated silica gel (Percoll). *Immunology*, **40,** 133.

Gleich G.J., Frigas E., Loegering D.A., Wasson D.L. & Steinmüller D. (1979) Cytotoxic properties of the eosinophil major basic protein. *J. Immunol.* **123,** 2925.

Gleich G.J., Loegering D.A., Kueppers F., Bajaj S.P. & Mann K.G. (1974) Physiochemical and biological properties of the major basic protein from guinea pig eosinophil granules. *J. exp. Med.* **140,** 313.

Gleich G.J., Loegering D.A. & Maldonado J.E. (1973)

Identification of a major basic protein in guinea pig eosinophil granules. *J. exp. Med.* **137,** 1459.

GLEICH G.J., LOEGERING D.A., MANN K.G. & MALDONADO J.E. (1976) Comparative properties of the Carcot-Leyden crystal protein and the major basic protein from human eosinophils. *J. Clin. Invest.* **57,** 633.

GOETZL E.J., WELLER P.F. & VALONE F.H. (1979) Biochemical and functional bases of the regulatory and protective roles of the human eosinophil. In: *Advances in Inflammation Research* (Ed. by G. Weissman, B. Samuelsson and R. Pasletti), Vol. 1, pp. 157. Raven Press.

HALLBERG T., GURNER B.W. & COOMBS R.R.A. (1973) Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int. Arch. Allergy appl. Immunol.* **44,** 500.

KAY A.B. (1979) The role of the eosinophil. *J. Allerg. clin. Immunol.* **64,** 90.

MCLAREN D.J., MCKEAN J.R., OLSSON I., VENGE P. & KAY A.B. (1982) Morphological studies on the killing of Schistosomula of *Schistosoma mansoni* by human eosinophil and neutrophil cationic proteins *in vitro*. *Parasite Immunol.* (In press.)

MANCINI F., CARBONARA A.O. & HEREMANS J.F. (1965) Immunochemical quantitation of antiserum by single radial immunodiffusion. *Immunochemistry*, **2,** 235.

METCALFE D.D., GADEK J.E., RAPHAEL G.D., FRANK M.M., KAPLAN A.P. & KALINER M. (1977) Human eosinophil adherence to serum-treated Sepharose: granule-associated enzyme release and requirement for activation of the alternative complement pathway. *J. Immunol.* **119,** 1744.

OLSSON I. & VENGE P. (1974) Cationic proteins of human granulocytes. II. Separation of the cationic proteins of the granules of leukemic myeloid cells. *Blood*, **44,** 235.

OLSSON I., VENGE P., SPITZNAGEL J.K. & LEHRER R.J. (1977) Arginine-rich cationic proteins of human eosinophil granules. Comparison of the constituents of eosinophilic and neutrophilic leukocytes. *Lab. Invest.* **36,** 493.

SPRY C.J.F. (1980) Eosinophilia and hypereosinophilic syndromes. *Trans. Royal Soc. trop. Med. Hyg.* **74** (suppl.), 3.

TAI P.C. & SPRY C.J.F. (1976) Studies on blood eosinophils. I. Patients with a transient eosinophilia. *Clin. exp. Immunol.* **24,** 415.

VENGE P., ROXIN L.E. & OLSSON I. (1977a) Radioimmunoassay of human eosinophil cationic protein. *Brit. J. Haematol.* **37,** 331.

VENGE P., ZETTERSTRÖM O., DAHL R., ROXIN L.E. & OLSSON I. (1977b) Low levels of eosinophil cationic proteins in patients with asthma. *Lancet*, **8034,** 373.

WASSON D.L., LOEGERING D.A., SOLLEY G.O., MOORE S.B., SCHOOLEY R.T., FAUCI A.S. & GLEICH G.J. (1981) Elevated serum levels of the eosinophil granule major basic protein in patients with eosinophilia. *J. Clin. Invest.* **67,** 651.

WINQVIST I., OLSSON I., WERNER S. & STENSTAM M. (1981) Variations of cationic proteins from eosinophil leukocytes in food intolerance and allergic rhinitis. *Allergy*, **36,** 419.