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THEORETICAL ASPECTS OF THE EOSINOPHIL GRANULOCYTE

By

H. HORTLING
Helsingfors, Finland

In 1846, certain leukocytes were found to contain granules of a coarser kind than those previously observed, and in 1878 Ehrlich was able to stain these granules red with eosin. Thus the study of the role of the eosinophil leukocyte began. In 1914 a monograph on the eosinophil cell, including 2758 references, was published by Schwartz.

Certain facts relating to the eosinophil cell were already regarded as established some ten years ago, namely, that it is a particular type of cell developing from a stem cell in the bone marrow, that the eosinophil cell in the blood probably is the same as that in the tissue, and that this cell is somehow associated with the protein molecule and with immunological processes.

During the last decade, interest was focussed on the eosinophil cell for two reasons. Firstly, the method of direct count in the wet chamber—first described in 1888—gained general acceptance after the publication, in 1946, of a thesis by the Norwegian Rud and, two years previously, of Randolph's description of the phloxine staining. Secondly, the intensive study of the adrenocortical hormones brought the observation that hormones of the cortisone group are capable of causing eosinopenia in the peripheral blood. This observation was first reported in 1948 by Thorn et al. of Boston.

The view that the eosinophil cell is a particular type, produced in the bone marrow, is still the one most commonly held

(Homma 1921, Gross 1954). The total life-span of this cell has been estimated at 6 days. In the circulating blood it lives for 2 days (Essellier et al. 1951–52). On eosinotactic stimulation, the eosinophil cell is capable of migrating to organs such as the lungs, the connective tissue, the gastrointestinal canal, the lymph nodes and serous cavities (Homma 1921, Romani 1952, Teir et al. 1955), where it probably is disintegrated in tissues belonging to the reticuloendothelial system (Essellier & Wagner 1952). Local accumulation of eosinophil cells occurs within a few hours, and no mitoses are observable. By contrast, it is believed that formation of lymphocytes may occur in the tissues, since a local accumulation of these cells requires several days and cell mitoses have been observed (rev. by Speirs & Wenck 1957). It should be mentioned, however, that Godlowski observed extramedullary formation of eosinophils in the small intestine of dog (1952, 1953).

In order to differentiate the causes of the variations in number of the eosinophil cells, it seems worth while to attempt to distinguish between factors stimulating, or otherwise causing, cell formation and factors with an inhibitory effect on the cell count. It is difficult, however, to be consistent in this respect.

When parasites, micro-organisms and foreign material such as polysaccharides and protein substances are administered to certain animal species, an increase in the eosinophil cell count results not only in the blood but also locally at the site of administration of the foreign substance and in the peritoneal fluid (Hayes & Dougherty 1954, Speirs et al. 1956). This reaction is unspecific and may be elicited with non-antigenic material. If the experimental animal has previously been immunized with an injected antigen, the local accumulation of eosinophils is much stronger, up to 50 per cent of the total cell count. This effect is, thus, specific and may be produced with a large number of substances with antigenic properties (comp. Speirs et al. 1957).

Histamine and similar substances, which are associated with the disintegration of tissues in inflammatory conditions, are,

perhaps, in part responsible for the initial, unspecific increase in eosinophils. Histamine cannot, however, bring about any persisting increase in the number of eosinophils, and its effect on the cells in the peripheral blood in man is ambiguous (Sampbell 1943, Kalaja 1946, Romani 1952, Vaughan 1953, Redd & Vaughan 1955). A marked increase in the eosinophil cell count may occur without any simultaneous increase in histamine in the blood. By contrast, injection of pollen extract, *i.e.* of polysaccharides, causes a persisting increase in eosinophils (Speirs 1955–56).

Adrenaline should, perhaps, also be listed among the substances causing an increase in the number of eosinophils. In experiments performed by the present writer and Pekkarinen (1949), the eosinophil count of human beings gradually increased by 70 per cent, on average, during a one-hour infusion of adrenaline in a physiologically possible concentration. When the infusion was interrupted, the increase was followed by the characteristic eosinopenia. Simultaneously with the eosinophils, the other granulocyte forms also increased in number, amongst them the immature granulocytes, which seems to indicate that cell formation or a release from the bone marrow was responsible also for the increase in the number of eosinophils. In adrenalectomized rats and mice several investigators have reported a similar increase in eosinophil granulocytes after injection of adrenaline or following other stresses such as cold (Speirs & Meyer 1949, Mathieu de Fossey 1950, Jakobson & Hortling 1954). It has not been clarified, however, whether the response to adrenaline here described is associated with the other eosinophil-increasing factors mentioned above. This point would, perhaps, be worthy of study. An increase in the eosinophil cell count is also brought about by inhibition of the reticuloendothelial system, which prevents the disintegration of these cells (Esselier, Marti & Morandi 1954).

Of the factors exerting an inhibitory influence on the eosinophil cell count in the blood, mention should firstly be made of the adrenocortical hormones, which belong to the glucocorti-

coid group. We know that one to two hours after the administration of cortisone, the number of circulating eosinophils decreases both in man and experimental animals, just as after administration of ACTH. This decrease in the peripheral blood is probably often due to increased migration of these cells to various tissues, for instance the lungs and the mucous membrane of the gastrointestinal canal (Godlowski 1953, Mundy 1954, Wegelius & Teir 1957). After administration of cortisone, the eosinophila in the peritoneal fluid exhibits, however, variations in the cell count resembling those observed in the blood (Higgins 1953, Speirs & Wenck 1956). It has also been shown that eosinophilic lung infiltrations disappear under the influence of ACTH (Essellier & Koszewski 1951). It seems probable that an inhibition of the release of cells from the bone marrow is a contributory cause of the cortisone eosinopenia in the blood (Jeanneret & Essellier 1957). This conclusion is corroborated by the observation that after administration of cortisone, the eosinophil elements in the bone marrow do not decrease. By contrast, the latter seem to increase in number simultaneously with the occurrence of eosinopenia in the peripheral blood. Only a protracted influence of ACTH brings about a decrease in the cell count in the bone marrow (Rosenthal *et al.* 1950, Uhrbrand 1954). A return to normal eosinophil values in the blood results from increased bone marrow activity (Durgin & Meyer 1951). The observation that cortisone does not cause disintegration of the eosinophil cell *in vitro* (Hudson 1954) is in conformity with what has been said above. In addition, Essellier *et al.* have recently (1957) emphasized that the effect of cortisone on the eosinophils is dependent on the proportion of the latter that are juvenile. If the proportion of juvenile eosinophil cells is high, a larger dose of cortisone and more protracted administration are required in order to bring about eosinopenia.

In regard of the effect of cortisone deficiency, *i.e.* of adrenalectomy, on the eosinophil cells of experimental animals the results of different investigators do not always agree. In mice, the local eosinophil response to injected non-toxic antigen is

much stronger and more persistent in adrenalectomized animals than in normal animals (Panzenhagen & Speirs 1953). However, in recent investigations by Wegelius & Teir, the local eosinophilia in rat's stomach was not increased, as compared with normal animals, in untreated adrenalectomized animals or in adrenalectomized animals pretreated with massive doses of atrophine. After adrenalectomy, an increase in the eosinophil count was observed in rats (Jakobson & Hortling 1954). It is possible, however, that in this case the same effect of stress was involved as may be produced with adrenaline and cold in adrenalectomized animals.

The role of the adrenal cortex in the 24-hour variation of the eosinophil cell is well known (Mathieu de Fossey & Deltour 1950, Halberg *et al.* 1951, Jakobson & Hortling 1952, Kaine *et al.* 1955).

The effect of cortisone on the eosinophil cell can be abolished by inhibition of the reticuloendothelial system (Essellier & Wagner 1952). Under certain experimental conditions ascorbic acid (Backus 1951, Rümke 1954) and heparin (Godlowski 1952, Muerhcke *et al.* 1952) also inhibit the effect of adrenaline or cortisone.

The inhibitory effect of adrenaline on the eosinophil granulocyte is better understood than the stimulating effect already discussed. It may be regarded as established that when adrenaline causes eosinopenia, it primarily affects the tissue cells causing an increase in their need for cortisone, instead of acting via the adreno-pituitary system, as was previously assumed. It seems more likely that adrenaline inhibits the hormone production of the adrenal cortex, since eosinopenia fails to appear if the experimental animal has been pretreated with adrenaline, but not if ACTH is previously administered (Vershoff 1957). In man, an adrenaline eosinopenia which disappeared after adrenalectomy and hypophysectomy was reelicited by pretreatment with cortisone (Kark & Muerhcke 1952, Hortling & Björkesten 1956). In addition, it has proved typical for the adrenaline eosinopenia that it is not inhibited by Nembutal (Keuskamp *et al.* 1957), whereas this

drug inhibits the eosinopenia brought about with salicyl preparations. Thus, it has to be admitted that the ultimate cause of the eosinopenia brought about with adrenaline is not yet understood.

Endocrine glands other than the adrenals, or their hormones, do not seem to play any major part in the regulation of the eosinophils. Mention may, however, be made of the contentions that the growth hormone is capable of increasing the cell count in the blood (Fruman & Gordon 1956), that the oestrogenous hormones can, for a short time, inhibit an eosinopenia due to pain (Louwerens & Smelik 1954), and that thyroid hormone probably increases the cell consumption (Shrewsbury Jr. & Reinhardt 1955).

Obviously the spleen, also, influences at least the eosinophil cells in the blood to some degree, probably via the bone marrow. In certain animal species splenectomy causes an increase in the eosinophil cell count. This effect does not, however, seem to be associated with the effect of the adrenocortical hormones, considering that there is no difference between normal and splenectomized animals with regard to the occurrence of adrenaline eosinopenia, and that splenectomy does not cause any further rise in the increased eosinophil count observed in rats after adrenalectomy (Dury 1950, Jakobson & Hortling 1954, 1954).

The function of the eosinophil cell is not yet fully understod. The investigations of Speirs & Wenck (1957) already mentioned elucidate this point from a new aspect. These writers recently showed that cortisone inhibits the local accumulation of eosinophils consequent upon reinjection of antigen. They regarded this as evidence that the eosinophil cell is associated with antibody formation, since massive doses of cortisone, which they used in their experiments, are capable of inhibiting the formation of antibody although they have little or no effect on the circulating antibodies or on the antigen-antibody reactions. Furthermore, roentgen doses known to inhibit antibody formation also prevent local accumulation of eosinophils. Speir's observations seem, thus, to suggest that

local eosinophil cell accumulation is more probably associated with the formation of antibody than with antigen-antibody reactions, breakdown of unspecific protein or shock reactions, as was previously assumed. The writers in question contend that the eosinophil granulocyte exerts the effect described above by enzymatically binding certain antigens which are later phagocytized by macrophages and utilized by them in the formation of antibodies. Previously, it was mainly the mononuclear cells that were credited with a role in antibody formation. Hence there is, perhaps, reason to point out that at least in some experimental animals the lymphocytes, for instance, and the eosinophil cells do not always react in the same way to disturbances in the equilibrium of the adrenocortico-pituitary system (Jakobson & Hortling 1954). In addition, it was found that after pretreatment with cortisone of hypophysecomized patients with mammary cancer, adrenaline again produced the eosinopenic effect which had vanished after the operation, whilst the lymphocyte response to adrenaline was not influenced in the direction of a further decrease in the cell count when the patients were pretreated with cortisone (Hortling & Björkesten 1956).

In completion of this review a few remarks may be made regarding the structure and chemical composition of the eosinophil cell and its granules. On disintegration of eosinophils, Charcot-Leyden crystals are often seen, which are believed to originate in the nucleus. The granules are characterized by crystallinic structures, which appear to develop from cytoplasmic vesicles (Braunsteiner & Pakesch 1957). The granules seem to be composed of a phospholipid with a protein nucleus and to contain, in addition, oxyribonucleic acid and, perhaps, iron (Wintrobe 1952). The protein substance contains an abundance of basic arginine, which has an antihistamine effect (Vercauteren *et al.* 1952). The eosinophil cell seems also, however, to contain a quantity of histamine corresponding to one-third of the amount contained in normal blood, whilst it has been reported that the basophil granulocyte contains one-half of the total amount of the histamine in

the blood (Graham *et al.* 1955). It is also possible that the eosinophil cell is capable of causing detoxification by enzymatic disintegration via oxidation (Vercauteren 1953). This cell also has a certain capacity of phagocytizing foreign substances (Mesnil 1895, Kärki *et al.* 1939).

This rather fragmentary survey has shown that the eosinophil granulocyte still constitutes a fascinating problem, and that its function and role have not yet been convincingly elucidated.

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