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Peroxidase and phospholipid deficiency in human eosinophilic granulocytes – A marker in population genetics

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Summary. A large scale investigation was carried out in order to establish the frequency of peroxidase and phospholipid deficiency of eosinophilic granulocytes among various ethnic groups in Israel. The simplicity of the method for staining eosinophilic peroxidase renders it a useful marker for the study of population genetics.

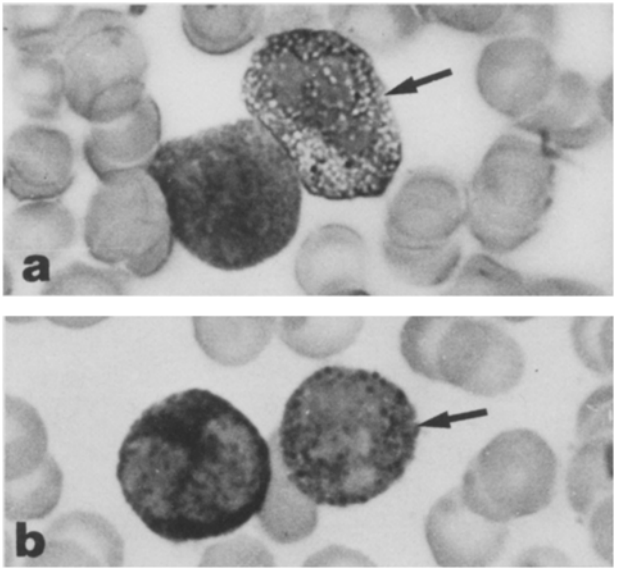
A 'new' hereditary defect of human eosinophilic granulocytes, consisting of complete absence of phospholipid and peroxidase staining was described in 1968 by Presentey[1]. The anomaly did not seem to be associated with particular clinical symptoms. The first 2 cases were encountered in families of Yemenite Jews[2] and an additional case was found in an Iranian Jew[3]. Family studies suggested an autosomal recessive mode of inheritance. The 1st population survey showed that the trait was not rare among Yemenite Jews; there were decreasing frequencies in Iraqi, Iranian and North-African Jews[4], but no cases were found among Ashkenazi of Central and East European origin, nor among Sepharadi Jews of the Balkans. A further study[5] disclosed the occurrence of the eosinophilic defect among Israeli Arabs of the Galilee, with a frequency close to that found among Iraqi, Iranian and North African Jews[4]. Since then, the study has been continued on a large scale, including a total of 69,133 people. The distribution of the affected individuals according to ethnic groups and the respective gene frequencies are presented in the table.

The anomaly was detected by screening the blood smears of all patients sent to a regional laboratory for routine hematological examinations, using specific peroxidase staining of the eosinophils according to Undritz[6] and staining for phospholipids according to Lison[7] (fig.a and b). Smears showing negative staining with these methods were subsequently subjected to nonspecific peroxidase staining according to Graham-Knoll[8] in order to ascertain that the anomaly did not affect the neutrophils. Special care was taken to identify family links between positive cases for further genetic studies.

The overall results of the study carried out during the period 1970–1976 are summarized in the table. They enclose the findings out of a total Jewish population of 63,465 individuals living in the Rehovot district and grouped according to ethnic origin as well as those obtained from the study of 1182 from the Galilee region. As shown in the table, the highest frequency of affected individuals as well as that of the mutant gene was found among Yemenite Jews, followed in decreasing order by Jews of North-African and Iraqi-Persian extraction and by the Arab population studied. So far, the eosinophilic anomaly has not been detected among Sepharadi Jews of the Balkans. Its rare occurrence among Ashkenazi Jews (L:11,325) let us presume that sporadic cases would be found also among non-Jewish European populations, whose genetic make-up is close to that of Ashkenazi Jews.

One such case has been already reported in the literature[9] and an additional case was incidentally detected by us in a Swedish gril residing temporarily in Israel.

Out of the 88 cases carrying the anomaly, 14 had family links and 74 were unrelated. The mode of inheritance was compatible with an autosomal recessive character of the mutant gene as previously suggested. Moreover, 14 individuals exhibited a partial loss of enzyme activity, manifested by a weak peroxidase staining of the eosinophils. Their



a) Positive peroxidase staining of neutrophilic and eosinophilic (arrow) granulocytes of a normal patient. b) Positive peroxidase staining of neutrophilic granulocytes and negative staining of eosinophilic granulocyte (arrow) of a deficient patient.

Frequencies of affected people and carriers among various ethnic groups in Israel

Ethnic groups	No. of randomly investigated individuals	No. of affected affected individuals	Frequency of affected individuals (%)	Frequency of carriers (2 pq) (%)	Frequency of genes q	±SE pq/N
Jewish						
Yemenites	11,212	44	0.39	11.7	0.0626	0.0016
Iraqi, Iranian	7,746	13	0.17	7.9	0.0410	0.0016
North Africans	10,532	26	0.25	9.5	0.0500	0.0015
Ashkenazi	33,975	3	0.008	0.02	0.009	0.003
Arabs	1,182	2	0.17	7.9	0.0411	0.0041

parents were unaffected, as were those of the individuals with complete absence of eosinophilic peroxidase. In no instance were the 2 types of deficiencies observed in the same families.

It is not certain whether a mutation of a regulatory gene is responsible for the intermediate activities of peroxidase[3]. Additional possibilities related to structural aspects of the enzyme need further investigation. Using chemical methods for more accurate characterization of enzyme properties and activities, one can expect to identify additional mutant alleles and eventually to detect sporadic cases of double heterozygous for different mutant alleles.

So far, no deleterious effects attributable to the lack of eosinophilic peroxidase could be observed in affected individuals and it seems that they are able to react with increased eosinophilic counts, as seen by us in several patients.

Recently Presentey[10] showed that the eosinophilic anomaly was expressed also at the ultrastructural level, by an enlargement of the core of the granules, and by an extreme thinning of the cortex, which is known to be the site of peroxidase activity in normal eosinophils.

The importance of the eosinophilic anomaly described[4,5] resides in its usefulness as an additional genetic marker for the study of population genetics. It parallels to some extent the distribution of other mutant genes, like G-6PD deficiency and Thalassemia, common to populations of the Mediterranean area. At the historical-geographical level, the continuation of these studies, especially among non-Jewish populations of this area, may help to trace affinities between different ethnic groups as well as to explain variety within populations. It should be mentioned in this context that the staining method of Undritz used by us is technically very simple and can be substituted for the standard methods used for staining of peripheral blood smears, thus allowing the detection of the anomaly while performing routine differential counts.

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Liposomes as immunological carriers for the preparation of antimannosyl antibodies

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Summary. Antiserum was raised against an aminophenyl derivative of D-mannose grafted on to a liposomal surface. As characterized by immunodiffucion, quantitative precipitation and hapten inhibition, the antiserum was found to contain mannose specific antibodies in addition to antibodies against the aromatic phenyl group.

The production of antibodies towards saccharide haptens generally requires the conjugation of saccharides with carrier proteins like bovine serum albumin or keyhole limpet hemocyanin[2,3]. In this communication we describe a new method for eliciting anticarbohydrate antibodies using liposomes as carriers for the monosaccharide, D-mannose, thereby eliminating the need for removing carrier-specific antibodies since liposomes themselves are known to be poorly immunogenic[4].

Methods. D-Mannose was covalently coupled to multilamellar phosphatidylethanolamine liposomes (egg lecithin:cholesterol:dicetyl phosphate:phosphatidylethanolamine = 7:2:1:2, molar ratio) as p-aminophenyl-α-glycoside using glutaraldehyde as the coupling reagent as described earlier[5]. Mannosylated liposomal suspensions (30 mg lipid/ml and 6 mg sugar/ml) were emulsified with an equal volume of complete Freund's adjuvant and 2 ml of the emulsion was injected into the hind food pads of a single rabbit. Three injections were given at every 10 days of which the last 2 were given in incomplete Freund's adjuvant. 7 days after the last injection, antiserum was collected by cardiac puncture. For immunological characterization of the antiserum, synthetic conjugates were prepared by coupling p-aminophenyl-α-D-mannopyranoside and p-aminophenyl-β-D-galactopyranoside with bovine serum albumin (BSA) through water soluble 1-ethyl-3-(dimethylaminopro-