A GENETIC DEFECT IN "ACQUIRED" AGAMMAGLOBULINEMIA*

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Phytohemagglutinin (PHA) has been shown to effect biochemical and morphologic changes in the small lymphocytes of normal human peripheral blood.\(^1\)\(^2\)\(^7\) The percentage of morphologic transformation of small lymphocytes to blastlike cells on exposure to PHA is reduced in a variety of conditions associated with abnormalities of the lymphoid system, such as Hodgkin's disease, sarcoidosis, and chronic lymphocytic leukemia.\(^3\)\(^4\) Recent studies from our laboratory have demonstrated that the in vitro, PHA-induced incorporation of labeled precursors into DNA and RNA by lymphocytes is significantly diminished in cells of adult "acquired" agammaglobulinemic individuals when compared to the incorporation by normal control cells. This difference between agammaglobulinemics and normals is independent of the culture-medium serum supplement (bovine, normal human, or autologous), implicating a cellular abnormality in the agammaglobulinemic patient.\(^5\)

In an effort to investigate further the possible genetic aspects of this disease, the in vitro response of peripheral blood lymphocytes obtained from the parents of such patients was studied in our assay systems.\(^6\) An autosomal recessive mode of inheritance can be inferred.

Methods.—The study group included 4 adult patients (ages 30-45) with "acquired" agammaglobulinemia whose levels of serum immunoglobulins G, A, and M were less than 10% of normal; a 21-year-old male with repeated infection but normal serum immunoglobulin levels (a second cousin of a known adult "acquired" agammaglobulinemic, see Discussion); 7 parents (ages 55-75) of the patients, whose serum immunoglobulin levels were normal; and 23 normal control individuals (ages 25-65), of both sexes, with normal serum immunoglobulin levels. None of the individuals studied were receiving medications.

Peripheral venous blood was collected in heparin (heparin sodium, Upjohn Company, Kalamazoo, Michigan), and the erythrocytes were separated by gravity sedimentation, in 16 × 125-mm screw-cap tubes, for 2 hr at room temperature. When necessary, blood specimens from agammaglobulinemic patients were gently centrifuged to facilitate sedimentation. The leucocyte-rich plasma supernatant was aspirated and the lymphocyte concentration determined. The leucocyte suspension was then centrifuged, and the pellet was washed in Eagle's minimal essential medium (MEM) Spinner salts solution and resuspended in culture medium at 350,000 lymphocytes/ml. Three-ml aliquots of each cell suspension were dispensed into 16 × 150-mm rubber-stoppered tubes, so that each culture tube contained approximately 1 × 10⁶ lymphocytes. The cells were then cultivated as described previously.\(^8\)

The culture medium consisted of MEM Spinner medium supplemented with L-glutamine, MEM nonessential amino acids, penicillin (50 units/ml), streptomycin (50 mcg/ml), and 10% bovine serum. Culture medium ingredients were obtained from Grand Island Biological Company, Grand Island, New York. A single lot of bovine serum was used in all studies.

PHA-M (control 485336; Difco Laboratories, Detroit, Michigan) was used as the mitogen. Each vial, dissolved in 5 ml Spinner salts, was diluted 1:10, and 0.1 ml of this dilution was added to each of the PHA-stimulated cultures at the appropriate time. Control cultures received an equivalent volume of diluent.

Separate assays were performed to test the incorporation of Cⁱ⁴-thymidine (Tdr-2-Cⁱ⁴)
into DNA and H³-uridine (Udr-5-H³) into RNA. The DNA assay system used is that described by Tormey and Mueller,⁶ and the RNA assay system of Mueller and Le Mahieu⁷ was modified by the substitution of H²-uridine for H³-cytidine as the RNA precursor (Fig. 1).

Results.—The leucocyte responses to PHA, gauged by the incorporation of labeled precursors into DNA and into RNA, are expressed in terms of cpm/1 × 10⁶ lymphocytes. The responses of the normal controls, the agammaglobulinemic patients, and their parents were segregated into three respective groups for evaluation. Similar observations were made in both the DNA and the RNA assay systems; the incorporation values of the patients and of the parents were lower, for the most part, than those of the controls (Fig. 2). Also, the range of response of the patients and the parents was more restricted than that of the normal controls, who exhibited a comparatively wide range of incorporation values.

Because of the wide range demonstrated by the normal controls, the findings were statistically analyzed by the nonparametric H-test of Kruskal and Wallis for one-way rank analysis of variance.⁸ H-test evaluation demonstrated that the PHA responses of the patients and their parents were distinguishable from those of the normal controls, with a significance of p < 0.001.

When a patient was compared with his respective parent or parents, it was evident that sex linkage was not involved. The abnormality in response to PHA was observed in both parents of two male patients, as well as in the mothers of two male patients and in the father of a female patient.

Discussion.—The extremely high incidence of qualitative and/or quantitative aberrations in immunoglobulins in the first-degree relatives of patients with “acquired” agammaglobulinemia, studied by us ten years ago, led us to suggest that this disorder is genetically determined.⁹ Additional evidence for the genetic nature of idiopathic “acquired” agammaglobulinemia was provided by the report by Wollheim et al.¹⁰ that showed consanguinity in distant relatives of patients with this disorder and the presence in the same family of two patients with adult “acquired” agammaglobulinemia.

The fact that both parents of our patients, when available for testing, showed an abnormal in vitro response to PHA indicates an autosomal mode of inheritance. Clinically, the presumed abnormal allele carried by the parents appears to be recessive and to express itself in the homozygous patients only.
As far as the in vitro assay is concerned, the abnormal gene seems to express itself as fully in single dose as in double dose, unless one assumes that the parents of the patients had a higher activity when they were the same age as the patients. The relationship of the assay results and the clinical syndrome of "acquired" agammaglobulinemia remains to be elucidated.

The findings obtained in a second cousin of a patient with "acquired" agammaglobulinemia lend further support to the concept that this condition is a genetically determined abiotrophy. This family (37 members) studied by us ten years ago contained many individuals with serologic abnormalities. The patient was found to have agammaglobulinemia at age 21; the second cousin (IV-13; see pedigree, ref. 9), now age 21, has had repeated infections during
the past two years. His immunoglobulin levels are currently normal; however, the response of his lymphocytes in this assay system was considerably impaired as were the responses of his parents. These findings suggest that abnormalities in lymphocyte metabolism may precede the decline in immunoglobulin levels.

These studies were also performed in some of the patients and their parents with purified lymphocyte suspensions prepared by glass wool column separation methods. In such preparations, at least a 99 per cent purity of lymphocytes was obtained. The results obtained with the purified lymphocytes were comparable to those with leucocyte-rich plasma. Furthermore, in the DNA assay system, similar observations were made without the use of an amethopterin block, although the magnitude and kinetics of the response are under further investigation.

There are several possible explanations for the observed biochemical abnormalities. The peripheral blood lymphocytes are composed of a heterogeneous population of cells from different tissue sites, with varying life spans, and have been exposed to different antigenic stimuli. Whether the defective response of cells from agammaglobulinemias and their parents reflects an alteration in subpopulations of lymphocytes, increased susceptibility of these cells to death in vitro, or an alteration in the kinetics of the in vitro response is currently being studied.

Several genetically determined human disorders, e.g., Huntington's chorea, are known to first manifest clinical symptoms late in life. Such diseases are transmitted by autosomal genes, and diagnosis can be made only when symptoms appear and not by laboratory tests. Thus, neither the presymptomatic subject nor the carrier can be identified by current methods. The demonstration of abnormal lymphocyte metabolism in the parents of patients with "acquired" agammaglobulinemia, however, provides a means for the detection of asymptomatic carriers. Furthermore, with this method, subjects may be detected before their immunoglobulins fall to subnormal levels. To our knowledge, this is the first example of a laboratory test that detects the carrier state in a genetically determined disorder that becomes manifest in adult life. It seems reasonable that other diseases of unknown etiology with clinical onset in adult life may have a genetic basis and that laboratory tests will be developed for the detection of presymptomatic patients as well as asymptomatic carriers, thus proving a genetic basis. "Acquired" agammaglobulinemia may be a prototype of such diseases.

Summary.—The in vitro responses of lymphocytes to PHA, as gauged by the incorporation of C14-thymidine into DNA and H3-uridine into RNA, were found to be significantly diminished in agammaglobulinemic patients and their asymptomatic parents, compared to those in a group of normal controls. The findings strongly suggest that "acquired" agammaglobulinemia is a genetically determined disorder.

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