Familial Immunopathy With Chromosomal and Functional Abnormalities of Lymphocytes

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Lymphocytes from members of a family with multiple immunologic disorders had abnormal chromosomes and defective in vitro function. The clinical findings ranged from agammaglobulinemia in the propositus to periarteritis nodosa in his grandnephew. The wife of the propositus was clinically healthy, but her lymphocytes were hyporeactive when cultured with nonspecific mitogens. The high incidence of lymphocyte abnormalities in the children may be the result of the mating of two individuals who have abnormal lymphocytes.

THE CAUSE OF IMPAIRED IMMUNOGLOBULIN synthesis in antibody deficiency diseases is unknown. A genetic abnormality underlies sex-linked agammaglobulinemia,[1] and family studies indicate that idiopathic "acquired" agammaglobulinemia (IAA) may be due to recessive autosomal genes.[2,3] The presence of abnormal chromosomes in some patients with an isolated deficiency of IgA[4,7] is further evidence of a genetic disturbance. Recent investigations suggest that RNA synthesis[8] and DNA transcription[9] are defective in the lymphocytes of patients with IAA, but not all are in agreement with this interpretation.[10]

This report describes a remarkable family in which multiple immunologic disorders were found in three generations. The propositus has IAA, and his wife, although clinically normal, has abnormal lymphocytes. The high frequency of immunologic disturbances in the children is due, we believe, to the fortuitous mating of two individuals with abnormal lymphocytes.

MATERIALS AND METHODS

Chromosome Studies

Short-term cultures of peripheral blood were done by the Moorhead method.[11] Forty to 50 metaphases from each culture were examined, and the chromosomes of 9–16 karyotypes were counted and analyzed. Gaps, breaks, and acentric fragments were classified as structural changes. The per cent of metaphases with numerical or structural abnormalities was compared with an analysis of the chromosomes of 23 normal subjects, as well as with published data from other laboratories.[12,14] Up to 13% of the metaphases with less than 46 chromosomes, up to 0.6% of the metaphases with more than 46 chromosomes, and structural changes not exceeding 12% were considered to be normal.[15]

In Vitro Studies of Lymphocytes

Lymphocytes were obtained from heparinized blood and separated as described by Bach and Hirschhorn.[16] Cell suspensions (1.5×10^6 cells/ 1.5 ml tissue culture) consisting of

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Submitted January 27, 1971; revised August 2, 1971; accepted August 3, 1971.

Supported by USPHS Grant No. AM-5610-03.

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about 90% mononuclear cells were cultures for 3 or 6 days. To the 3-day cultures was added 0.01 U of phytohemagglutinin-M (PHA; Difco); 0.01 ml of rabbit antihuman lymphocyte serum (ALS), 100 U of Stroptokinase-Streptodornase (SKSD; Lederle), 5 U *Monilia albicans* extract (Hollister-Stier Laboratories), or 10 μg tuberculin (PPD) was added to the 6-day cultures. ^{3}H-Thymidine (specific activity, 6.7 Ci/m*M*, 1 μCi/1.5 ml) was added 3 hr before termination of the culture. DNA was extracted with 5% trichloroacetic acid, washed with absolute methanol, dissolved in 1/10 *N* NaOH, added to Bray's solution, and counted in a liquid scintillation counter for 30 min. Results were expressed in two ways: the total ^{3}H-thymidine uptake and the index of response. The latter was calculated by dividing the ^{3}H-thymidine uptake at the time of a maximum response by the ^{3}H-thymidine uptake of the unstimulated control cultures. No response gives an index of 1.

Complement

Total serum complement was measured by the method of Wasserman and Levine,[17] and C3 levels were estimated on radial immunodiffusion plates (Hyland). C_{1q} determinations were carried out by Dr. H. Müller-Eberhardt, Scripps Clinic and Research Foundation, La Jolla, Calif.

ϒ-Globulins

Serum levels of IgG, IgA, and IgM were measured on radial immunodiffusion plates (Hyland).

CASE REPORTS

A.N., the propositus, is a 48-year-old man of Italian descent. The diagnosis of hypogammaglobulinemia was established in his case at the age of 44, when he was referred because of multiple bacterial infections for 14 yr. There was no evidence of a malignant lymphoma or of malabsorption. Plasma cells were absent from the bone marrow. He was challenged with keyhole limpet hemocyanin (KLH) and failed to develop either delayed hypersensitivity or circulating antibodies to that antigen. However, the tuberculin test was positive. ϒ-Globulin substitution therapy was started in 1966, and he has remained well since then. His mother had severe rheumatoid arthritis.

MnN., the 20-yr-old daughter of A. N., developed classical rheumatoid arthritis at the age of 19. She is treated with chloroquin and aspirin and is in remission. Her serum immunoglobulin levels are normal.

T.N., the 6-yr-old daughter of A.N., developed severe rheumatoid arthritis at the age of 4 yr. Aspirin therapy resulted in a moderate improvement. Her serum immunoglobulin levels are normal.

R.N., the 3-yr-old daughter of A. N., is said to have had mumps twice and has had recurrent infections of the upper respiratory tract. No IgA was detected in her serum, and she has mild neutropenia and moderate lymphocytosis.

L.V.II., the 13-year-old son of A.N.'s nephew, has had recurrent urinary infections since the age of 10. At the age of 10 he developed the Stevens-Johnson syndrome and severe rheumatoid arthritis. Periarteritis nodosa developed when he was 12 yr, and treatment with corticosteroids was instituted. His serum immunoglobulin levels are normal.

The remaining members of the family are clinically normal. However, Pa.N., the 23-yr-old son of A.N., and J.V., the 67-yr-old sister of A.N., have consistently elevated serum IgG levels. The family tree is shown in Fig. 1.

RESULTS

Chromosome Analysis

A high incidence of structural and numerical changes was found in the chromosomes of the propositus (A.N.), five of his children (E.N., Pa.N., Mn.N., J.N., and T.N.), and his grandchild (G.N.) (Table 1). Thirteen per cent of the fathers metaphases had typical structural abnormalities including

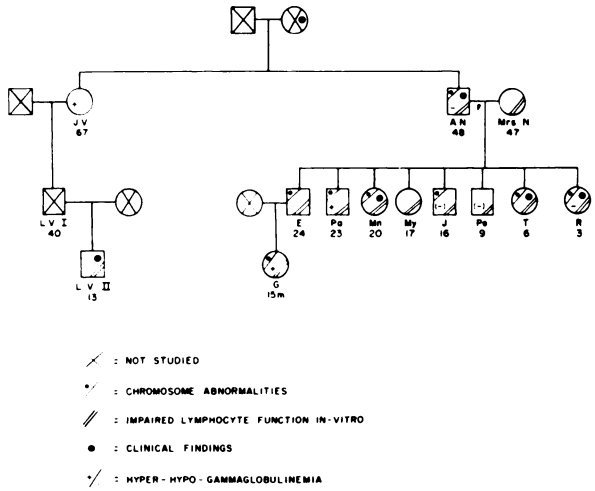

Fig. 1. Family tree.

fragments, gaps, and breaks. In addition, two karyotypes showed a clumped chromosome in the F group (Fig. 2). This unusual configuration in the F group also occurred in E.N. (17% of the karyotypes), Pa.N. (25%), Mn.N (10%), My.N. (22%), J.N. (10%), T.N. (20%), R.N. (20%), and in the grandchild G.N. (10%). The chromosomes of R.N., the daughter with the IgA deficiency, also had a smiliar configuration in the E group (13%), but we do not know whether this is related to the F group abnormality. Interestingly, she also had two karyotypes with monosomies in the E series. Chromosomes of the children E.N., Pa.N., J.N., T.N., and the grandchild G.N. had a high incidence of other aberrations, and there was a trisomy in the E series. Mn.N. displayed three monosomies of chromosome No. 18 in the E group. Two of these had simultaneously a monosomy in the F group. The chromosomes of the mother and siblings My.N., Pe.N., and R.N. were within normal limits. Chromosomes of J.V., L.V.I., and L.V.II were not studied.

Lymphocyte Function In Vitro

The lymphocytes of all members of the family were abnormal (Tables 2 and 3). In unstimulated cultures, the lymphocytes of both parents incorporated abnormally low amounts of ^{3}H-thymidine. The lymphocytes of both parents responded poorly to PHA, and in neither case was there a response to ALS. Judging by the amount of ^{3}H-thymidine incorporated, the lymphocyte abnormality was more severe in the father than in the mother. The cells of the mother reacted normally in the presence of specific mitogens. The lymphocytes of A.N. failed to respond to SKSD and *M. albicans;* there was also no response to PPD, despite a positive tuberculin skin test.

Two children, J.N. and T.N., had lymphocytes that were also hyporeactive in either resting cultures or in cultures containing mitogens. J.N.'s cells did not respond to ALS, SKSD, *Candida,* or PPD, but did respond to PHA; T.N.'s cells were unreactive to *M. albicans* and PPD but were reactive in the presence of PHA, ALS, and SKSD. One child, Pe.N., had lymphocytes that responded only to PHA.

Table 1. Chromosome Analysis

	Total Metaphases Analyzed	Numerical Changes				Structural Changes	Total Metaphases Karyotyped	Abnormal F
		<46	46	>46	Polyploid			
Control*		<13%		<0.6%		<12%		
A. N.	40	3 (8%)	37	0	0	5 (13%)	10	2 (20%)
Mrs. N	40	2 (5%)	38	0	0	1 (2.3%)	10	0
E. N.	40	3 (8%)	34	3 (8%)	0	1 (2.3%)	12	2 (17%)
Pa. N.	50	5 (10%)	42	3 (6%)	0	8 (16%)	16	4 (25%)
Mn. N	40	7 (18%)	32	0	1	5 (13%)	10	1 (10%)
My. N.	42	1 (2.3%)	39	0	1	3 (8%)	9	2 (22%)
J. N.	40	9 (23%)	28	2 (5%)	1	2 (5%)	10	1 (10%)
Pe. N.	40	0	40	0	0	4 (10%)	11	0
T. N.	40	7 (18%)	33	1 (2%)	0	9 (23%)	15	3 (20%)
R. N.	40	5 (13%)	35	0	0	0	15	3 (20%)
G. N.	50	6 (12%)	42	2 (4%)	0	8 (16%)	10	1 (10%)

*Control values based on 23 normal persons.

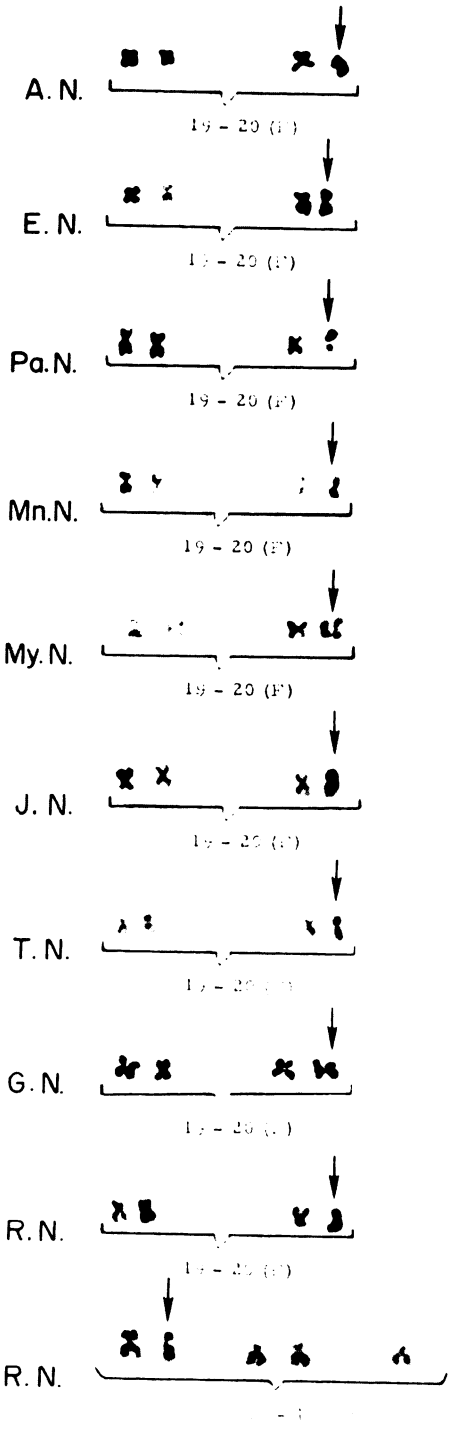

Fig. 2. Karyotypes of ten members of N family. Arrow points to abnormal chromosome of each subject.

The lymphocytes of five children (E.N., Pa.N., My.N., R.N., Pe.N.) incorporated significantly higher amounts of ^{3}H-thymidine in unstimulated cultures than did those of normal subjects. Despite this high spontaneous

Table 2. Lymphocyte Responses to Nonspecific Mitogens

	RESTING 3d	RESTING 6d	PHA(CPM)	PHA(INDEX)	ALS(CPM)	ALS(INDEX)
CONTROL	2,477.67· 260.48	1,733.21· 214.41	148,165.13· 11,408.46	74.92· 2.95	3,690.9 · 778.85	2.34· 0.61
A.N.	977.36· 153.38*	761,83· 188.75	62,812.54· 27,622.09*	64.27	372.66· 19.08*	0.49
Mrs. N	1,568.83· 294.94	1,290.73· 122.26	95,591.74· 10,611.09*	60.93	593.74· 425.28	1.18
E.N.	627.51· 731.28*	17,080.47· 782.31*	352,523.39· 21,629.56*	76.18	--	--
Pa.N.	2,506.92· 98.6	10,362.51· 531.64*	155,745.52· 26,292.76	44.41	4,394.22· 547.37	0.42
Mn.N.	1,821.33· 656.74	1,211.12· 424.74	165,321.61· 30,045.36	76.56	4,697.23 1,351.17	3.88
My.N.	4,072.23· 213.36*	2,070.13· 72.41	151,035.39· 30,943.5	37.09	3,608.21· 34.71	1.74
J.N.	1,557.72· 229.68	1,460.62· 651.39	167,095.35· 29.941.57	107.27	1,196.36· 678.37*	0.82
Pe.N.	2,357.49· 84.21	3,877.36· 91.09*	213.832.19· 39,197.31	90.7	2,621.87· 646.7	0.68
T.N.	2,159.34· 344.73	766.19· 8.69*	149,169.13· 12,853.25	69.08	2,076.57· 281.59	2.68
R.N.	3,677.69· 102.32	1,512.56· 77.34	130,598.49· 10,912.6	35.51	926.91· 370.99*	0.61
G.N.	12,790.09·1,562.61*	4.093.66·2,449.05*	584,107.94· 19.853.94*	45.67	--	--
L.V.II	2,553.43· 31.88	1,174.99· 159.42	322,274.66· 74,626.65*	126.21	7,354.08· 707.22*	6.26

· standard error [ ] p = 0.05 [*] p = 0.01

*** Control values based on results in 12 normal persons. Significance levels calculated by Student's t test for unpaired data using four replicate cultures.**

activity, the cells of Pa.N., My.N., and R.N. were less responsive to PHA, ALS, and *M. albicans* than expected, as indicated by a significantly decreased index of response. My.N.'s and R.N.'s lymphocytes did not respond to SKSD, but Pa.N.'s did. The lymphocytes of E.N. were peculiar. As compared to the values obtained with 24 normal persons, they incorporated relatively large amounts of ^{3}H-thymidine in unstimulated cultures. Despite their higher background of DNA metabolism, these cells responded to mitogens as actively as control cells. The lymphocytes of E.N.'s daughter, G.N., also incorporated significantly excessive amounts of ^{3}H-thymidine in resting cultures and after exposure to PHA. Her in vitro reaction to SKSD and *Candida* was normal. The cells of Mn.N. were also hyperactive in the presence of SKSD.

The lymphocytes of L.V.II, the nephew with periarteritis nodosa, were also unusual. Their behavior in unstimulated cultures was normal but they incorporated significantly large amounts of ^{3}H-thymidine in the presence of PHA, ALS, and SKSD.

Serum Studies

The globulin and complement levels are shown in Tables 4 and 5.

DISCUSSION

The family we have described is remarkable for several reasons. Immunologic disease, in one form or another, runs through it for three generations.

Table 3. Lymphocyte Responses to Specific Mitogens

	SKSD (CPM)	SKSD(INDEX)	CAND. (CPM)	CAND. (INDEX)	PPD(CPM)	PPD(INDEX)
CONTROL	5,386.12± 747.6	3.36± 0.67	2,230.29± 156.13	2.36± 0.34	4,471.03± 932.64	2.49± 1.09
A.N.	991.65± 157.82*	1.3	1,021.92± 297.06	1.34	639.5 ± 261.98*	0.84**
Mrs.N.	4,181.63± 976.92	3.24	3,743.93± 721.82	2.9	1,458.26± 171.63	1.41**
E.N.	58,365.67± 17,484.12*	3.28	12,555.55± 1,036.24	0.71	188.3 ± 51.4 *	0.01
Pa.N.	30,001.35± 10,316.83*	2.9	11,633.31± 986.31*	1.12	353.98± 55.14*	0.03
Mn.N.	197,834.88± 20,720.71	163.35	1,799.89± 383.79	1.49	1,818.02± 537.09	1.5
My.N.	1,309.06± 41.64*	0.63	2,172.28± 558.52	1.05	2,687.09± 217.58	1.3**
J.N.	799.39± 235.68*	0.55	1,417.68± 47.72*	0.97	556.82± 743.55*	0.38
Pe.N.	593.26± 230.78*	0.15	2,802.98± 146.95	0.72	764.99± 233.21*	0.2
T.N.	2,483.18± 1,060.68	3.2	691.5 ± 121.2	0.89	434.2 ± 151.88*	0.56
R.N.	476.31± 76.42*	0.31	1,672.12± 142.18	1.11	430.36± 154.82*	0.29
G.N.	5,178.67± 866.8	1.27	3,221.83± 176.07	0.79	--	--
L.V.II	90,001.26± 11,049.63	76.6	2,209.87± 226.92	1.88	649.13± 142.69*	0.55**

± standard error | p = 0.05 | * p = 0.01 | ** skintest positive

*** Control values based on results in 12 normal persons. Significance levels calculated by Student's t test for unpaired data using four replicate cultures.**

The kinds of disease range from severe hypogammaglobulinemia in the propositus to periarteritis nodosa in his grandnephew. Two daughters have rheumatoid arthritis, and a third, with repeated sinopulmonary infections, is lacking IgA.

Another interesting feature of this family is that Mrs. N., a healthy woman, has abnormal lymphocytes. They were repeatedly hypoactive in unstimulated cultures and hyporesponsive to nonspecific mitogens. Her lymphocytes did, however, respond normally to specific mitogens. Since the possibility of any blood relationship between the parents has been ruled out, we believe the high incidence of immunologic abnormalities in their children can be explained by the fortuitous mating of two individuals with abnormal lymphocytes. The occurrence in all eight children of defects due to a genetic factor present in only one parent has a probability of 2^8, which seems highly unlikely.

Lymphocyte dysfunction, ranging from profound hyporeactivity of A.N.'s lymphocytes to marked hyperreactivity of those of E.N., was found in most members of the family. The behavior of lymphocytes after stimulation with PHA is of particular interest when compared with their activity in unstimulated cultures. A normal ^{3}H-thymidine uptake in the latter, but an abnormally increased uptake after PHA stimulation (as in the case of L.V.II, who has

Table 4. Immunoglobulin Levels in mg/cc Measured on Immunodiffusion Plates in 1965 and 1969

	1965			1969		
	IgG	IgA	IgM	IgG	IgA	IgM
Standard	(6-12)	(0.7-2.0)	(0.4-1.0)	(5-14)	(0.5-4.9)	(0.3-2.6)
A. N.	1.6	<0.1	<0.1	1.3	0	0
Mrs. N	–	–	–	12.0	2.0	2.0
E. N.	–	–	–	10.5	2.7	1.1
Pa. N	13.5	0.95	0.52	16.0	1.6	1.2
Mn. N.	17.0	2.1	0.67	14.0	1.85	1.2
My. N.	19.5	1.85	0.67	8.4	2.0	1.1
J. N.	17.0	0.55	0.52	11.0	0.6	0.45
Pe. N.	13.5	1.2	0.36	8.4	1.05	0.4
T. N.	15.0	0.32	0.85	14.0	1.1	1.5
R. N.	–	Not born	–	11.0	0	0.4
G. N.	–	Not born	–	19.0	0.14	0.6
J. V.	22.0	2.1	1.6	16.0	1.85	2.2
L. V. II	–	–	–	6.8	0.92	1.95

periarteritis nodosa) suggests the presence of "labile" lymphocytes with abnormal sensitivity to certain mitogens. The combination of increased ^{3}H-thymidine uptake in resting cultures, but normal incorporation after exposure to PHA (Pa.N., My.N., and R.N.), indicates that relatively few cells of the population are stimulated by this mitogen.

In addition to these findings, the propositus, five of his children, and his grandchild had abnormal chromosomes. It is reasonably certain that the abnormalities were present in their lymphocytes, because the technique for their detection involves examination of cultured blood cells that are stimulated to divide by PHA. It is unlikely that the findings of aneudiploidy and nonspecific but similar structural changes in the chromosomes of A.N. and

Table 5. Complement Levels

	Clq* μg/ml		C3 1-2 mg/ml	CH 50 150-250 U
Standard	190 ± 10	100 ± 1	1.2-1.7	147-250
A.N.	167	92	2.3	185
Mrs. N.	260	145	2.2	192
E. N.	–	–	1.8	156
Pa. N.	227	125	3.0	–
My. N.	193	106	1.4	172
Mn. N.	238	131	1.9	111
J. N.	194	107	1.75	161
Pe. N.	156	86	2.1	147
T. N.	322	177	2.2	167
R. N.	242	133	2.0	192
G. N.	–	–	1.0	–
J. V.	230	128	2.9	–
L. V. I	180	100	3.0	–
L. V. II	194	107	2.2	–

*Two standards were used for determination of C_{1q}.

five of his eight children are a coincidence. The numerical abnormality of the E group of chromosomes (trisomy) may have particular significance because trisomy rarely occurs in normal individuals. The structural abnormality of a chromosome in the F group (chromosomes number 19 and 20) may be of litle consequence as an isolated finding, but this was present in the father and seven of his children. The abnormal appearing chromosome found in these eight persons was never seen in studies of 23 normal subjects. The normal chromosome pattern of the mother suggests that the chromosome abnormalities were transmitted from the father.

This is not the first instance in which chromosome abnormalities have been associated with immunologic deficiency. Abnormal chromosomes have recently been found in patients with isolated IgA deficiency,[4-7] although not in all cases. The 18th chromosome is generally involved, either by a deletion of its long arms or by the formation of a ring chromosome. Sporadic examples of chromosome abnormalities have also been reported in ataxia teleangiectasia and the "Swiss" type of thymic aplasia.[1]

The correlation frequently observed between cases of immunologic deficiency diseases and autoimmune disorders is of particular interest. Patients with agammaglobulineamia can develop rheumatoid arthritis, dermatomyositis, scleroderma, vasculitis, or autoimmune hemolytic anemia.[18] Moreover, in certain families, such as the N. family, some members have hypogammaglobulinemia and others an autoimmune disease. These unexpected associations must have some meaning, for they now seem more than a statistical quirk. Is it conceivable that both the deficiency syndrome and the autoimmune disease are different expressions of a more basic defect? The results in the N family suggest this because of the multiple abnormalities of their immunocompetent cells. The cellular defect in agammaglobulinemia has been attributed to defective transcription of genetic information by an abnormal messenger RNA.[19-21] Our results, which are the first to demonstrate abnormalities in both chromosomes and function of lymphocytes, also suggest abnormalities of genetic material.

Finally, C_{1q}, a globulin component of the complement system with γ-mobility, has been found deficient in patients with agammaglobulinemia. The deficiency is less severe in IAA[22] than in congenital agammaglobulinemia.[1,23] The results of A.N., a decreased serum concentration of C_{1q}, an increased level of C3, and a normal titer of total hemolytic complement, are similar to those of other patients with IAA.[24,25] In some members of the N family the pattern was similar, but in others C_{1q} or C3 was increased. The meaning of these disturbances in the complement system is not understood at present. However, their concordance with quantitative abnormalities in the γ-globulins suggests a basic alteration common to the two systems of immunoproteins.

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