Laboratories of Cytology and of Medical Genetics, Cleveland Psychiatric Institute, Cleveland, Ohio; and Department of Medicine, Euclid Clinic Foundation, Euclid, Ohio

Antinuclear Antibody Factors and Nuclear Staining in Mothers of Children Affected with Down's Syndrome

Freddie L. Pollard, Steven Zsako, Margaret A. Kelsall Arnold R. Kaplan

SUMMARY

Sera from 50 mothers of G_1-trisomy affected children and from 50 age-matched mothers, whose offspring included no chromosomal-aneuploidy affected children, were tested for presence of antinuclear antibody factors and nuclear staining with human squamous epithelium and with mouse liver. Consistently, more of the mothers of G_1-trisomy affected children included positive findings in both tests, compared to the age-matched mothers.

Various types of antinuclear antibodies occur at relatively high frequencies in the sera of patients affected with systemic lupus erythematosus, systemic rheumatic disorders and other diseases (Hasker et al, 1965; Seligmann et al, 1965; Ritchie, 1967). The antinuclear antibodies are immunologic gamma-globulins which act upon nucleoproteins, DNA, or other nuclear constituents. The chromosomal abnormalities of Down's syndrome as noted by Fialkow (1964, 1966, 1968) and Burgio et al (1965) have also been related to the abnormal production of thyroid autoantibodies in mothers of children with Down's syndrome. Vallotin and Forbes (1966) stated that all nine tested members of a group of women with *menopausa praecox* showed antinuclear antibody factors to cytoplasm of rabbit ova. They hypothesized that such factors might be associated with infertility as well as with production of children affected with chromosomal abnormalities.

The purpose of this study was to determine the incidence of antinuclear factors in blood samples from mothers of children affected with Down's syndrome and from age-matched mothers of children with normal karyotypes.

Material and Methods

Tests for antinuclear factors were made on blood sera from 50 mothers of G_1-trisomy affected children and 50 age-matched mothers of children having normal karyotypes. Serum from a patient having untreated systemic lupus erythematosus and human control serum (Colorado Serum Company) were used with each batch of staining to determine the specificity of the fluorescence. Mouse liver was used as the nuclear antigen in the indirect

Rec. 20. I. 1970

Acta Genet. Med. Gemellol. (1970), 19: 529-533

fluorescent antibody method described by Svec et al (1967); and human squamous cell epithelium was used in the indirect fluorescence method of Marmont et al (1967).

The undiluted serum was incubated with the antigen for 30-40 minutes at 37°C in a moist chamber. After two washes with buffered saline solution for 5 minutes, the slides were incubated with F.I.T.C.-labeled antihuman globulin diluted to ¼ with buffered saline, washed again with buffered saline, and covered with 50% glycerol. Nonspecific staining was reduced by absorption of the labeled antisera through mouse powder (purchased from the Colorado Serum Company). A Zeiss photomicroscope with ultraviolet illumination from an HBO 200 (Osram) mercury burner, filtered through excitation *filter BG*12 and barrier *filter* 50, was used to determine fluorescence.

Results and Discussion

A great majority of the examined cells were positive for nuclear staining, in the mouse liver studies, for 3 of the 50 mothers of G_1-trisomy affected children and for only 1 of the 50 age-matched mothers. In the human squamous epithelial studies, a great majority of the examined cells were positive for 5 of the mothers of G_1-trisomy affected children and for 3 of the age-matched mothers (Tab. I).

The determination of positive or negative results is arbitrary, and varies with different investigators. We have also tabulated the numbers of individuals in each category, for each test, whose slides were observed to show any nuclear fluorescent staining. According to this criterion, 17 of the 50 mothers of G_1-trisomy affected children (34%) were positive in the mouse liver test and 28 (56%) were positive in the human squamous epithelial test. According to the same criterion, the age-matched group of 50 mothers included 8 who were positive in the mouse liver test (16%) and 17 (34%) who were positive in the human squamous epithelial test. Tab. II shows the distribution of individuals with observable nuclear staining.

Data based upon the standard criterion for a positive designation — i. e., a majority of examined cells being positive — are consistent with the suggestion that the mothers of G_1-trisomy affected children are more likely to show antinuclear antibody factors than the other mothers. The numbers of individuals involved, who showed majorities of their cells positive for fluorescent nuclear staining, however, are too small to facilitate statistical comparison. The two groups of mothers may be compared statistically, however, if the dichotomy is based solely upon the presence or absence of any observed fluorescent nuclear staining. The distributions of the two groups of mothers according to this broad criterion are shown on Tab. II. The mothers of the G_1-trisomy affected children include a higher proportion of positive individuals than the other mothers, for each test. A χ^2 analysis was made for the different distributions between the two groups of mothers, according to the dichotomy of presence or absence of any observed fluorescent nuclear staining. The different distribution according to the test with mouse liver yields $\chi^2 = 4.32$ (1 *df*), indicating a probability that the difference is due only to chance, $P < 0.05$. The different distribution according to the test with human squamous epithelium yields $\chi^2 = 2.72$ (1 *df*), indicating a probability that the difference is due only to chance, $P < 0.10$.

Tab. I. Antinuclear antibody factors

Antinuclear tests	G_1 mothers		Control mothers	
	+	—	+	—
Mouse liver	3	47	1	49
Human squamous epithelium	5	45	3	47

Tab. II. Nuclear staining - Incidence of positive (+) and negative (—) reactions

Antigen	Maternal age group							
	25—35		35—45		over 45		Total	
	+	—	+	—	+	—	+	—
Mouse liver								
G_1 mothers	5	6	3	10	9	17	17	33
Control mothers	1	10	4	9	3	23	8	42
Human squamous epithelium								
G_1 mothers	4	7	7	6	12	14	23	27
Control mothers	1	10	6	7	8	18	15	35

The different groups of data based on our small sample are consistent with each other and suggest that mothers of G_1-trisomy affected children, compared to age-matched mothers with no chromosomal-aneuploidy affected children, are significantly more likely to show antinuclear antibody factors or nuclear staining with mouse liver and with human squamous epithelium.

The indirect immunofluorescent Coons (1956) test, as modified by Beutner and Witebsky (1962) and Friou (1962), is a common diagnostic test for lupus erythematosus. The test is positive in 95-100% of patients with lupus erythematosus, in 3% of unselected males in the 20-60-year age range, in 7% of unselected females in the same age range, and in almost 50% of individuals over 80 years of age (Bauer et al, 1968).

The data in Tab. II indicate that the greatest contrast between the relatively high incidence of observable nuclear staining in the mothers of G_1-trisomy affected children and the age-matched mothers occurs in the youngest (i. e., 25-35 years) age group. This is the maternal age group in which occurrence of offspring affected with the G_1 trisomy is relatively most age independent.

In general, the occurrence of observable stain was transient. The great majority of subjects with a positive test based on one serum sample did not show a positive test in consecutively drawn serum samples. There were very few exceptions: only 2 of the 100 subjects showed observable nuclear staining with mouse liver tests in two consecutively drawn (one-month intervals) serum samples, and both of them were

mothers of G_1-trisomy affected children. Two of the mothers of G_1-trisomy affected children showed observable nuclear staining with squamous epithelium with all three serum samples, and three of the age-matched mothers showed positive results with two consecutive serum samples.

The mothers of G_1-trisomy affected children showed a higher incidence with observable staining, and also a higher proportion with more intense staining.

Positive test ratings ranged from "faint" to "maximum positive" as characterized with known positive lupus erythematosus serum. In both the mouse liver test and the squamous epithelium test, all of the positive samples from the age-matched mothers had low positive ratings (i. e., "faint", "small patch", and "+"). The only observed positive tests with higher ratings (i. e., "++", "+++", and "++++") were in sera from mothers of G_1-trisomy affected children: two in the mouse liver test, and three in the human squamous epithelium test. There was no evidence, in our small samples of subjects, of an association between positive results in the mouse liver test and positive results in the epithelium test.

Conclusion

These data indicate that the two groups of mothers are characterized by immunological differences, and that there is an association between observable nuclear staining and having had a child affected with G_1 trisomy. The data therefore suggest that observable nuclear staining is associated with immunological characteristics which affect a woman's predisposition to suffer G_1-chromosomal nondisjunction during ovogenic meiosis, and production of a G_1-trisomy affected child.

Acknowledgment. The authors appreciate the technical assistance of Dorothy Wolfert, R.N., and Ann Kontra, R.N. These studies have been supported in part with research grants from the Euclid Clinic Research Foundation and from the National Institutes of Health (G.R.S.-05563).

References

Bauer J. D., Ackermann P. G., Toro G. (1968). Bray's Clinical Laboratory Methods. 7th Ed. C. V. Mosby, St. Louis, Mo.

Beutner E. H., Witebsky E. (1962). Studies on organ specificity. XIV. Immunofluorescent studies of thyroid reactive autoantibodies in human sera. J. Immun., **88:** 462.

Burgio G. R., Severi F., Rossoni R., Vaccaro R. (1965). Mongolism and thyroid autoimmunity. Lancet, **1:** 166.

Coons A. H. (1956). In J. F. Dannielli: General Cytochemical Methods. Academic Press, New York.

Fialkow P. J. (1964). Autoimmunity: a predisposing factor to chromosomal aberrations? Lancet, **1:** 474.

— (1966). Autoimmunity and chromosomal aberrations. Amer. J. Hum. Genet., **18:** 93.

— Uchida I. A. (1968). Autoantibodies in Down's syndrome and gonadal dysgenesis. Ann. N.Y. Acad. Sci., **155:** 759.

Friou G. J. (1962). Fluorescent spot test for anti-nuclear antibodies. Arthritis Rheum., **5:** 407.

Hasker J., Mackay I. R., Miller J. J. (1965). The incidence of "antinuclear factor" in human disease. Aust. Ann. Med., **14:** 96.

Marmont A. M., Damasio E. E., Bertorello C., Rossi F. (1967). Studies on the antiperinuclear factor. Arthritis Rheum., **10:** 117.

Ritchie R. F. (1967). The clinical significance of titered antinuclear antibodies. Arthritis Rheum., **10:** 544.

Seligmann M., Cannat A., Hamard M. (1965). Studies on antinuclear antibodies. Ann. N. Y. Acad. Sci., **124:** 816.

Svec K. H., Blair J. D., Kaplan M. H. (1967). Immuno-pathologic studies of systemic lupus erythematosus. J. Clin. Invest., **46:** 558.

Vallotin M. B., Forbes A. P. (1966). Autoimmunity in gonadal dysgenesis and Klinefelter's syndrome. Lancet, **2:** 264.

Riassunto

La presenza di fattori anticorpali antinucleari e di colorazione nucleare è stata analizzata, con epitelio squamoso umano e con fegato di topo, in 50 madri di trisomici G_1 e 50 madri di controllo, della stessa età, con prole indenne da aneuploidia. Le madri dei trisomici hanno dato risposte positive ad ambedue i test più frequentemente delle madri di controllo.

Résumé

La présence de facteurs anticorpaux antinucléaires et de coloration nucléaire a été analysée, avec épithélium squameux humain et avec foie de souris, chez 50 mères de trisomiques G_1 et 50 mères de contrôle, du même age, dont les fils n'étaient pas atteints d'aneuploïdie. Des réponses positives pour les deux tests ont été obtenues plus fréquemment chez les mères des trisomiques que chez les mères de contrôle.

Zusammenfassung

Bei 50 Müttern von Kindern mit Trisomie G_1 und 50 Kontrollmüttern des gleichen Alters mit Kindern ohne Aneuploidie wurde das Vorhandensein von Antikörper-, Antinuklear-und kernfärbenden Faktoren mit menschlichem Schuppenepithel und Mäuseleber untersucht. Bei den Müttern der Trisomiekinder war das Ergebnis beider Tests häufiger positiv als bei den Kontrollmüttern.

F. L. Pollard, Laboratory of Cytology, Cleveland Psychiatric Institute, 1708 Aiken Avenue, Cleveland, Ohio 44109, USA.