

Abnormal Immune Responses of Bloom's Syndrome Lymphocytes In Vitro

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ABSTRACT Bloom's syndrome is a rare autosomal recessive disorder, first characterized by growth retardation and a sun-sensitive facial telangiectasia and more recently demonstrated to have increased chromosome instability, a predisposition to malignancy, and increased susceptibility to infection. The present report concerns the immune function of Bloom's syndrome lymphocytes in vitro. Four affected homozygotes and five heterozygotes were studied. An abnormal serum concentration of at least one class of immunoglobulin was present in three out of four homozygotes. Affected homozygotes were shown capable of both a humoral and a cellular response after antigenic challenge, the responses in general being weak but detectable.

Blood lymphocytes from Bloom's syndrome individuals were cultured in the presence of pokeweed mitogen. The cells had an impaired proliferative response and synthesized less immunoglobulin at the end of 5 days than did normal controls. In contrast, they had a normal proliferative response to phytohemagglutinin except at highest concentrations of the mitogen. In the mixed lymphocyte culture, Bloom's syndrome lymphocytes proved to be poor responder cells but normal stimulator cells. Lymphocytes from the heterozygotes produced normal responses in these three systems.

Disturbed immunity appears to be one of several major consequences of homozygosity for the Bloom's syndrome gene. Although the explanation for this pleiotropism is at present obscure, the idea was advanced that the aberrant immune function is, along with the major clinical feature—small body size, a manifestation of a defect in cellular proliferation.

INTRODUCTION

Bloom's syndrome (1-3) is a rare autosomal recessive disorder the major clinical features of which are severe growth retardation and a sun-sensitive telangiectatic erythema of the face. Increased chromosome breakage

and rearrangement are demonstrable in cultured cells and probably occur in vivo as well.

Two observations have raised the question of the adequacy of host-defense mechanisms in this disorder: (a) Most individuals with Bloom's syndrome present a striking history of infections during early life. This may explain the syndrome's relatively recent recognition as a clinical entity, after the advent of antibiotic therapy. Infections most often involve the respiratory and gastrointestinal tract and are caused by both gram-positive and gram-negative bacteria (unpublished observations). The severity and frequency of the infections tend to decrease with increasing age. Viral infections appear to be resisted normally. (b) Affected individuals are at an increased risk of developing malignant tumors at an early age; 4 of the first 5 persons recognized as having Bloom's syndrome and 8 out of the 50 known cases who have survived infancy have developed one or more malignant tumors. These observations prompted us to investigate immune function in individuals with Bloom's syndrome and their heterozygous parents.

METHODS

Affected homozygotes, heterozygotes, and controls. Four unrelated individuals with Bloom's syndrome were studied, one female and three males ranging in age from 2 to 23 yr. They are identified, as in reference 1, as 3 (HoCo), 32 (MiKo), 47 (ArSmi), and 50 (JeBl). The diagnosis was made on the basis of the classical clinical features and the finding of increased chromosome breakage in dermal fibroblasts, blood lymphocytes, or both. Five parents of three of the affected, ranging in age from 21 to 42 yr, were included in the study and will be referred to as "heterozygotes." 12 normal persons ranging in age from 2 to 47 yr served as controls. All subjects were clinically well and were not receiving medication at the time of the study.

Lymphocyte cultures. Leukocytes were isolated from venous blood by Ficoll-Hypaque gradient centrifugation (4); the preparations contained more than 85% small mononuclear cells. Cell yield was similar in persons with Bloom's syndrome, heterozygotes, and controls. For immunofluorescent studies, leukocytes were purified further by incubation at 37°C for 30 min with poly-L-lysine-coated carbonyl-iron

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in dextran (Technicon Instruments Corp., Tarrytown, N. Y.); phagocytic cells and free iron were removed with a magnet. Lymphocyte cultures were performed in triplicate in 0.2-ml volumes in flat bottom microtiter plates (Linbro, New Haven, Conn.). The culture medium was RPMI 1640 containing 100 U penicillin/ml, 100 μ g streptomycin/ml, 2 mM glutamine/ml (Grand Island Biological Co., Grand Island, N. Y.), and 10% pooled human AB serum. For mitogenic stimulation, 1.5×10^6 cells/0.2 ml were used; the appropriate concentration of mitogen was added in a volume of 10 μ l. Mixed lymphocyte cultures (MLC)¹ contained 1.5×10^6 responder cells and 3.0×10^6 irradiated (3,000 rad) stimulator cells in a volume of 0.2 ml. Included in each experiment were cultures without mitogen and cultures with autologous irradiated cells. Pokeweed mitogen (PWM) was purchased from Grand Island Biological Co., purified phytohemagglutinin (PHA) from Burroughs Wellcome Co., Research Triangle Park, N. C. PWM concentrations were expressed as dilutions of the manufacturer's stock solution, PHA concentrations as μ g/0.2 ml. Cultures containing mitogen were incubated in a 37°C humidified incubator (5% CO₂-95% air mixture) for either 78 or 126 h; MLCs were incubated for 126 h. 6 h before termination of the cultures, 1 μ Ci [*methyl*-³H]thymidine (sp act 2 Ci/mmol, New England Nuclear, Boston, Mass.) in a volume of 2 μ l was added to each well. Cell cultures were processed for liquid scintillation counting as previously described (5, 6).

In vitro immunoglobulin synthesis after PWM stimulation. 5×10^6 lymphocytes were cultured in 5 ml of medium RPMI 1640 with 10% human AB serum. The final concentration of PWM was 1:100 of the stock solution. After 5 days the cells were washed three times in minimal essential medium (MEM) without leucine, suspended in 1 ml MEM without leucine containing 5% fetal calf serum and 20 μ Ci [³H]leucine (sp act 30-50 Ci/mmol, New England Nuclear), and incubated for 4 h at 37°C. The cells were then centrifuged for 10 min at 1,500 g; the supernatant medium was removed and retained for further analysis. The cell pellet was lysed by addition of 1 ml of 0.5% Triton-X in phosphate-buffered saline (PBS), pH 7.2. The supernatant medium and cell pellet lysate were each centrifuged for 1 h at 20,000 g, and insoluble material discarded. Immunoglobulin (Ig) synthesis was determined by specific Ig precipitation of radiolabeled proteins (7, 8). 0.1 ml of supernatant medium or detergent-solubilized cell pellet lysate was mixed with 10 μ l of Ig class-specific antiserum (or 10 μ l of normal rabbit serum in controls) and incubated at room temperature for 30 min. The immune complexes were precipitated with a goat anti-rabbit Ig antiserum in equivalence. The immunological precipitate was washed three times with 0.1% Triton-X in PBS, centrifuged for 10 min at 1,500 g at 4°C, and then dissolved in 0.5 ml of 0.25 N acetic acid. Total protein synthesis was measured as counts per minute in the fraction precipitated by 5% trichloroacetic acid (TCA) with 20 μ l of human serum as carrier protein. The TCA precipitate was washed three times as above and then suspended in 0.5 ml of 0.25 N acetic acid. Radioactivity was determined by liquid scintillation counting. Further de-

¹Abbreviations used in this paper: Ig, immunoglobulin; MEM, minimal essential medium; MLC, mixed lymphocyte culture; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PPD, purified protein derivative; PWM, pokeweed mitogen; SI, stimulation index; TCA, trichloroacetic acid.

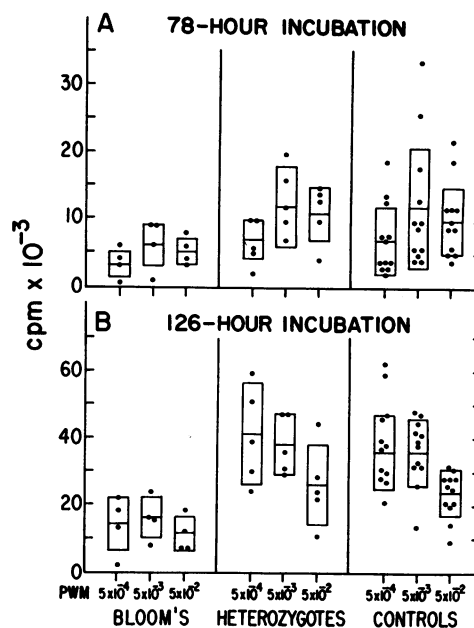


FIGURE 1 Lymphocyte stimulation by PWM. Lymphocytes from 4 persons with Bloom's syndrome (Bl), 5 heterozygotes (Hz), and 12 normal controls (NI) were cultured with three different concentrations of PWM. [³H]thymidine uptake was measured after incubation for 78 h (A) and 126 h (B). Bars denote mean \pm 1 SD. Note different scales for ordinates in A and B. PWM concentrations are expressed as dilutions of stock solution.

Statistical comparison of experimental groups:

Fig. 1A

Bl vs. NI	PWM 5×10^{-4}	$P < 0.05$
	PWM 5×10^{-3}	$P < 0.05$
	PWM 5×10^{-2}	$P < 0.025$

Bl vs. Hz	PWM 5×10^{-2}	$P < 0.025$
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Other comparisons, including Hz vs. NI, nonsignificant.

Fig. 1B

Bl vs. NI	PWM 5×10^{-4}	$P < 0.0025$
	PWM 5×10^{-3}	$P < 0.0005$
	PWM 5×10^{-2}	$P < 0.0025$

Bl vs. Hz	PWM 5×10^{-4}	$P < 0.01$
	PWM 5×10^{-3}	$P < 0.0025$
	PWM 5×10^{-2}	$P < 0.025$

Hz vs. NI, nonsignificant.

tails of the method will be described elsewhere (T. H. Hütteroth and S. D. Litwin, manuscript in preparation).

Combined assay for membrane Ig-bearing and sheep red cell rosette-forming lymphocytes. This was performed as described previously (6).

Skin testing. The following antigens were injected intradermally, and the reaction was observed at 24 and 48 h: mumps (Eli Lilly and Company, Indianapolis, Ind.), purified protein derivative (PPD) 1:10,000 (Connaught Medical Research Labs., Toronto, Canada), histoplasmin (Parke-Davis & Company, Detroit, Mich.), trichophyton 1:100 (Hollister-Stier Laboratories, Spokane, Wash.), and monilia 1:10 (Hollister-Stier Laboratories).

Naturally-occurring antibodies. Isohemagglutinin-titers were performed by Mr. W. L. Marsh, The New York Blood Center.

Active immunization. Three of the affected individuals were injected with tetanus toxoid (Lederle Laboratories, Pearl River, N. Y.) and monovalent influenza vaccine type B (Wyeth Laboratories, Philadelphia, Pa.). Blood was taken before and 3 wk after a single injection of each antigen. Antitetanus titers were determined by hemagglutination by Dr. Richard Hong, Department of Pediatrics, University of Wisconsin Center for Health Sciences, Madison. The anti-influenza titers were determined by hemagglutination by Dr. Jerome Schulman, Department of Microbiology, Mount Sinai School of Medicine, New York.

RESULTS

Lymphocyte stimulation by PWM. The proliferative response to PWM was studied as a function of PWM concentration after 78- and 126-h incubation periods (Fig. 1). At 78 h the pattern of response was the same for the three test groups with a maximum response at 5×10^{-8} dilution of PWM. However, the magnitude of the proliferation differed, with lymphocytes from Bloom's

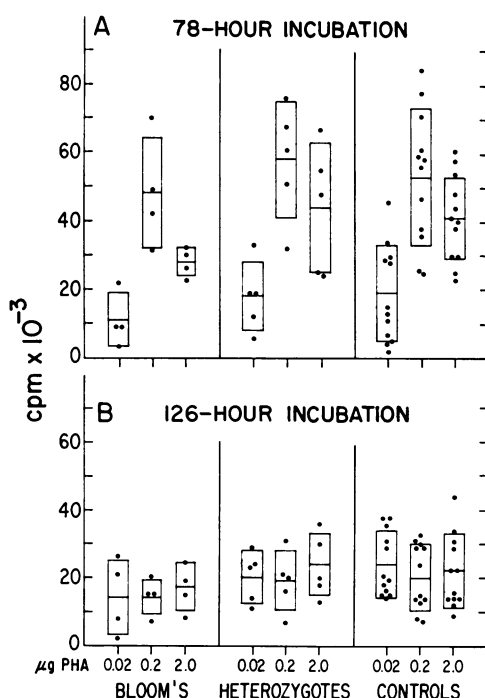


FIGURE 2 Lymphocyte stimulation by PHA. For experimental design, see legend of Fig. 1. PHA concentrations are expressed as µg/0.2 ml.

Statistical comparisons of experimental groups:

Fig. 2A

Bl vs. NI PHA 2.0 µg $P < 0.05$

Other comparisons, nonsignificant.

Fig. 2B

Group comparisons, nonsignificant.

TABLE I

Lymphocyte Reactivity of Individuals with Bloom's Syndrome, Heterozygotes, and Controls in MLC Testing

Lymphocyte combination	Responder lymphocytes	Stimulator lymphocytes	n	Mean cpm ± SEM (SI ± SEM)
a	Bl	NI _x	12	5,048 ± 1,180 (31 ± 8)
b	H _z	NI _x	15	10,847 ± 1,743 (24 ± 4)
c	NI	Bl _x	12	12,507 ± 3,908 (37 ± 7)
d	NI	H _z _x	15	11,207 ± 2,106 (38 ± 4)
e	NI	NI _x	24	14,733 ± 2,671 (46 ± 6)
f	Bl	Bl _x	4	187 ± 37
g	H _z	H _z _x	5	808 ± 215
h	NI	NI _x	12	309 ± 41

Combinations a through e represent pooled data from four different experiments. Cells from four individuals with Bloom's syndrome (Bl) and five heterozygotes (H_z) were each tested with three normal individuals (NI). Combinations f through h are base-line cultures in which responder cells were stimulated with their own irradiated leukocytes. n, number tested. The subscript x indicates irradiated cells.

syndrome individuals having lower responses than cells from normal individuals at all three concentrations of PWM. At 126 h the disparity between the magnitude of the response of Bloom's syndrome and control lymphocytes was even more pronounced. The above differences were statistically significant (see legend of Fig. 1). Heterozygotes and controls had similar results. Unstimulated cultures gave the following results after 78 and 128 h, respectively (±SEM): Bloom's syndrome, 129 ± 22 and 139 ± 94 cpm; heterozygotes, 280 ± 99 and 500 ± 242 cpm; controls, 184 ± 86 and 301 ± 78 cpm. These values were not subtracted from the counts obtained in mitogen-stimulated cultures.

Lymphocyte stimulation by PHA. The proliferative response to different concentrations of PHA was studied after 78- and 126-h culture periods (Fig. 2). After 78 h the maximum proliferation did not differ between cultures of lymphocytes from individuals with Bloom's syndrome, heterozygotes, and controls except at the highest concentration of 2 µg/0.2 ml of PHA, at which Bloom's syndrome individuals showed a lower response than controls (Fig. 2A). After 126 h the proliferative response did not differ between cultures from persons with Bloom's syndrome, heterozygotes, and controls (Fig. 2B) at any concentration.

The effect of the source of the serum used in the culture medium on proliferation of lymphocytes from Bloom's syndrome and control individuals was analyzed in two experiments using optimal concentrations of PWM (5×10^{-8} dilution) and PHA (0.2 µg). No differences in the response were observed when autologous

TABLE II
*Immunoglobulin and Protein Synthesis by Bloom's Syndrome and Control
Lymphocytes after PWM Stimulation*

	$\frac{\text{Ig cpm}}{\text{TCA cpm}} \times 100(\%) \pm \text{SEM}$			
	IgG	IgM	IgA	TCA
	<i>cpm \pm SEM</i>			
Culture medium				
Controls*	30.7 \pm 6.8	23.9 \pm 6.1	21.8 \pm 4.8	16,959 \pm 4,387
Bloom's syndrome				
3 (HoCo)	7.2	5.2	5.9	3,100
32 (MiKo)	2.3	1.1	0	5,508
47 (ArSmi)	5.3	6.4	3.1	2,496
Cell lysate				
Controls*	2.61 \pm 0.74	2.36 \pm 0.71	2.94 \pm 0.73	133,950 \pm 31,740
Bloom's syndrome				
3 (HoCo)	0.46	0.43	0.63	83,990
32 (MiKo)	0.35	0.15	0.38	119,084
47 (ArSmi)	0.24	0.38	0.18	79,316

* Mean of culture values from six control individuals.

serum was compared to homologous serum from normal persons.

MLC experiments. The ability of lymphocytes from persons with Bloom's syndrome to react to allogeneic cells was investigated in the one-way MLC (Table I). In each experiment, one person with Bloom's syndrome was studied together with one or two heterozygotes and three normal controls. To obtain suitable numbers for statistical analysis, the [^3H]thymidine-incorporation data of identical responder-stimulator combinations from all experiments were pooled and compared to other responder-stimulator combinations. The results demonstrated that lymphocytes from individuals with Bloom's syndrome responded less vigorously to normal stimulator lymphocytes than did normal responder lymphocytes to normal stimulator lymphocytes. The differences between the following groups were statistically significant (see Table I): combination *a* vs. *b*, $P < 0.01$; combination *a* vs. *d*, $P < 0.01$; combination *a* vs. *e*, $P < 0.0025$. Cells from heterozygotes and controls made similar responses to normal allogeneic cells (combination *b* vs. *e*). Bloom's syndrome cells stimulated normal cells as effectively as did control cells (combination *c* vs. *e*).

Combinations *f-h* of Table I represent base-line cultures in which responder cells were stimulated with autologous irradiated lymphocytes. Cells from persons with Bloom's syndrome had a lower spontaneous proliferation than did controls or heterozygotes. Stimulation indices (SIs) were calculated from these base-line culture data. "SI" is defined as counts per minute from the combination of responder cells *A* incubated with stimulator cells *B* divided by the counts per minute from

the combination of responder cells *A* and stimulator cells *A* ($\text{SI} = A\ B_*/A\ A_*$). When the different SIs were analyzed the response to normal allogeneic cells of Bloom's syndrome lymphocytes and control lymphocytes did not differ significantly (combination *a* vs. *e*), due in part to the spontaneous proliferation of Bloom's syndrome cultures being lower than controls (combination *f* vs. *h*).

Ig synthesis and secretion after PWM stimulation. PWM is known to induce Ig synthesis and secretion in lymphocytes from normal individuals (9). Here, lymphocyte cultures from the three persons with Bloom's syndrome tested synthesized less total cellular protein and had a lower percent Ig/TCA than control cultures (Table II). Parallel results were obtained from the medium in which the cells had been cultured. The decrease in cellular Ig synthesis and secretion affected all Ig classes.

Distribution of B and T lymphocytes in blood. The proportion of cells bearing membrane Ig (B lymphocytes), cells binding sheep red cells (T lymphocytes), and cells bearing neither marker ("null" lymphocytes) were determined in three affected homozygotes, two heterozygotes, and six controls (Table III). All individuals showed a normal distribution of B and T lymphocytes and a small percentage of null lymphocytes. The total blood leukocyte counts were in the normal range. The data suggest that a normal blood distribution of B and T lymphocytes existed in all three groups tested. It should be noted, however, that since the pooled antiserum had specificity for all of the major Ig heavy chain classes and light chain types, the data do not ex-

clude a distorted representation of the major Ig classes on the cell surface of peripheral blood lymphocytes.

Serum Ig concentrations. Serum concentrations of the major Ig classes were measured in the Bloom's syndrome homozygotes and heterozygotes (Table IV). One homozygote had a low IgM concentration and two had both decreased IgG and IgM concentrations. The fourth, 50 (JeBl), had a low IgA concentration, which is difficult to evaluate because of her young age. One heterozygote had a decreased concentration of IgM, and another had an elevated concentration of IgM.

Skin testing. Skin testing was performed on three of the persons with Bloom's syndrome. One individual, 47 (ArSmi), showed a positive response to mumps antigen (10 mm induration at 48 h), and a second person, 3 (HoCo), to both mumps (25 mm induration) and PPD (10 mm induration) at 48 h. The third showed no response to any of the antigens, but he had been shown earlier to be capable of sensitization with dinitrochlorobenzene.

Isohemagglutinin titers. Isohemagglutinin titers of the three persons with Bloom's syndrome were within the normal range.

Active immunization. Three of the persons with Bloom's syndrome were challenged with monovalent influenza type B vaccine and with tetanus vaccine (Table V). With the exception of 47 (ArSmi), who failed to show an increase in tetanus antibody, each increased his antibody titers; antibody titers fell within the low normal range.

TABLE III
Percentage of B, T, and Null Lymphocytes in Blood of Individuals with Bloom's Syndrome, Heterozygotes, and Controls

Source of cells	B lymphocytes	T lymphocytes	Null lymphocytes
Bloom's syndrome			
3 (HoCo)	12	88	0
32 (MiKo)	21	73	6
47 (ArSmi)	15	85	0
Heterozygotes*			
AKo	22	75	3
BKo	12	84	4
Controls			
1	19	74	7
2	18	79	3
3	15	77	8
4	21	76	3
5	11	84	5
6	23	77	0

B lymphocytes were identified by staining with fluorescein-conjugated anti-Ig antibody, T lymphocytes by their ability to bind sheep erythrocytes, and null lymphocytes as cells by their lack of either of these characteristics.

* Mother (AKo) and father (BKo) of 32 (MiKo).

TABLE IV
Serum Ig Concentrations in Affected Homozygotes and in Heterozygotes for the Bloom's Syndrome Gene

Source of serum	Age	Ig class*		
		IgG	IgA	IgM
	yr	mg/100 ml		
Bloom's syndrome				
3 (HoCo)	23	580	125	28†
32 (MiKo)	11	310†	22	12†
47 (ArSmi)	15	490†	23	9†
50 (JeBl)	2	760	15	35
Heterozygotes‡				
AKo	36	1,700	165	450†
BKo	35	1,550	74	97
JSmi	42	1,000	92	75
LBl	27	1,470	155	60
JaBl	20	1,370	180	31†

* Determined by radial immunodiffusion.

† Value outside mean ± 2 SD (27)

‡ AKo and BKo are parents of 32 (MiKo); JSmi is mother of 47 (ArSmi); LBl and JaBl are parents of 50 (JeBl).

DISCUSSION

Previous clinical observations of repeated bacterial infections in the majority of Bloom's syndrome patients and reports from several laboratories (10, 11) of decreased concentrations of serum Igs both had suggested the existence of an impairment of immune function in vivo in this rare genetic disorder. All four of the affected individuals in the present report have had repeated infections requiring antibiotics, three had definite decreases in serum Ig, one possibly had a low IgA. As adjuncts to our investigation of the immune response in vitro of Bloom's syndrome lymphocytes, the affected individuals were challenged with a panel of antigens, and it was shown that delayed hypersensitivity could be elicited and that a humoral immune response followed tetanus and influenza vaccination. It should be

TABLE V
Immunization of Three Persons with Bloom's Syndrome

Person immunized	Tetanus toxoid antibody titer		Influenza type B antibody titer	
	Pre-immunization	Post-immunization	Pre-immunization	Post-immunization
3 (HoCo)	64	512	80	320
32 (MiKo)	64	512	0	160
47 (ArSmi)	64	64	0	320

Each person was given one injection of vaccine and bled 21 days later. For tetanus immunization the titer range for controls up to 15 hr of age is between 512 and 1,024.

noted, however, that although the responses were present they were generally weak, and the limited data cannot rule out partial impairment.

In the present study, we have shown the following abnormalities *in vitro* in the blood lymphocytes of individuals with Bloom's syndrome: (a) In short-term culture with PWM there was an impaired proliferative response as measured by incorporation of radiolabeled thymidine and a decreased synthesis of Ig as determined by an immunoprecipitation assay. (b) In the one-way MLC, Bloom's syndrome lymphocytes are poor responder cells but normal stimulator cells.

The pattern of immune dysfunction encountered in Bloom's syndrome differs from that described for other immunodeficiencies and raises questions as to the nature of the inherited defect. The disturbances demonstrated could be on the basis of an abnormal B- or T-lymphocyte function, of abnormal processing or recognition of antigen, or of abnormality in some general activity such as cellular proliferation, an activity not restricted just to lymphocytes and the immune system.

Dysfunction of B lymphocytes is suggested by the poor responses made by the blood lymphocytes cultured with PWM. PWM has been found to stimulate predominantly B lymphocytes (12, 13), although a recent investigation has shown that both T and B lymphocytes will undergo mitosis when PWM is added to cell cultures in which both types of lymphocytes are present (14). When human lymphocytes are cultured in the presence of PWM, plasmacytoid cells develop (15) and cellular synthesis of Ig occurs (9). Dysfunction of Bloom's syndrome T lymphocytes is suggested by the results of the MLC experiments in which the proliferative responses of lymphocytes of persons with Bloom's syndrome and that of controls were significantly different when direct [³H]thymidine incorporation data in counts per minute, but not when SIs, were compared. Disagreement exists as to the best method of analyzing MLC data. In the present studies, if the SI is employed, an unusually low spontaneous incorporation of [³H]thymidine in Bloom's syndrome cultures leads to a distortion of the results, and for this reason, use of the absolute counts per minute of radioactivity seems preferable (16). The significance of the level of spontaneous DNA synthesis is unclear, but recent experimental data suggest that it is significantly correlated with the mitogenic response to the B-lymphocyte mitogen lipopolysaccharide and marginally correlated with the response to PWM but not correlated with the responses to the T-lymphocyte mitogens, PHA and concanavalin A (17).

A disparity exists between the findings on the one hand of a normal response to PHA (in other than the highest dosage), which suggests adequate T-lymphocyte function, and on the other hand of an impaired response in the MLC, which indicates defective T-lym-

phocyte function (18). The response in the MLC may be a more sensitive indicator of T-lymphocyte function than the PHA response, or the response in the MLC and that to PHA may assay different subpopulations of T lymphocytes. A similar aberration has been described in the Wiskott-Aldrich syndrome, in which a normal response to PHA is usually found along with decreased MLC reactivity (19). A dissociation in the opposite direction, PHA unresponsiveness and MLC responsiveness, has been described in human lymphoid cells from fetal liver (20) and in cases of combined immunodeficiency (21, 22). In Bloom's syndrome, evidence against an abnormal distribution in the blood of various lymphocyte subpopulations was obtained, normal proportions of B and T lymphocytes being demonstrable. At the present time, the disturbed immune function in Bloom's syndrome cannot be explained in terms of the known functions of lymphocyte subpopulations.

Chromosome instability ("breakage") and a predisposition to malignancy are found in at least three recessively transmitted human disorders, Bloom's syndrome, Fanconi's anemia, and ataxia telangiectasia. Xeroderma pigmentosum probably also should be included in this group of disorders (2). Two of these rare disorders, Bloom's syndrome and ataxia telangiectasia, and possibly xeroderma pigmentosum as well (23), also show immunological abnormalities. Both chromosomal abnormalities and immunologic impairment have been associated with human malignancies (3, 19). The latter, the immune deficiency, offers a direct explanation of the tendency of Bloom's syndrome individuals to develop malignancy prematurely, if the concept of immune surveillance is invoked. Support for immunologic surveillance has come from the predisposition to malignancy of persons with several forms of primary immunodeficiencies (19). Present information indicates that the immunologic abnormalities in ataxia telangiectasia involve cell-mediated immunity, with histologic changes in the thymus gland and frequently an absence of serum IgA, IgE, or both (19). A recent report has described defects in cellular immunity in xeroderma pigmentosum (23). It is clear that the pattern of immunologic abnormalities reported here in Bloom's syndrome is not the same as that in either ataxia telangiectasia or xeroderma pigmentosum; also, its pattern of chromosome aberrations is of a specific type (24, 25). In summary, despite the above provocative findings, no common denominator is yet apparent in these various genetic disorders, and no biological links between "chromosome breakage," impaired immune responses, and cancer can be identified at present.

It would seem reasonable at this point to speculate that although the genetic defect in each of these rare disorders just mentioned differs, they may all relate to the same biological function. There is evidence of im-

paired cellular proliferation in Bloom's syndrome. Growth retardation is a major clinical feature of the disease. Tissue cultures of dermal fibroblasts require longer periods of time between subculturing than do lines from most normal persons, and the cultures tend to have a short life span (J. German, unpublished observations). DNA chain elongation during the S phase of the cell division cycle in cultured skin fibroblasts from persons with Bloom's syndrome proceeds at a slower rate than in normal fibroblasts (26). Also, possibly related to a proliferative defect are our (T. H. Hütteroth and S. D. Litwin) unsuccessful attempts at establishing lymphoblastoid cell lines from Bloom's syndrome blood, despite a number of attempts using different culture conditions. The possibility should be considered that the immunologic abnormalities found in persons with Bloom's syndrome and other genetically determined disorders are secondary to a disturbance in cellular proliferation, with the specific pattern of immune defects dependent on the nature and severity of the proliferative defect and on the class of cells affected.

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