

Disorders of B Cells and Helper T Cells in the Pathogenesis of the Immunoglobulin Deficiency of Patients with Ataxia Telangiectasia

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ABSTRACT The pathogenesis of the immunoglobulin deficiency of 20 patients with ataxia telangiectasia was studied using an in vitro immunoglobulin biosynthesis system. 10 patients had no detectable IgA in their serum as assessed by radial diffusion in agar and 3 had a reduced serum IgA concentration. The peripheral blood mononuclear cells of 17 of the patients and 17 normal controls were cultured with pokeweed mitogen for 12 d and the immunoglobulin in the supernatants measured. The immunoglobulin synthesis was below the lower limit of the normal 95% confidence interval for IgM in 5 patients, for IgG in 8, and for IgA in 14. The mononuclear cells from 9 of the 10 patients with a serum IgA concentration < 0.1 mg/ml failed to synthesize IgA in vitro. None of the patients manifested excessive suppressor cell activity. All patients had reduced but measurable helper T cell activity for immunoglobulin synthesis by co-cultured normal pokeweed mitogen-stimulated B cells (geometric mean 22% of normal). Furthermore, the addition of normal irradiated T cells to patient peripheral blood mononuclear cells led to an augmentation of IgM synthesis in 15 of 17 and to increased IgG synthesis in 9 of the 17 patients studied, including 9 of the 12 patients who had synthesized IgG before the addition of the irradiated T cells. In addition, IgA synthesis was increased in all eight patients examined that had serum IgA concentrations > 0.1 mg/ml. These studies suggest that a helper T cell defect contributes to the dimin-

ished immunoglobulin synthesis. However, a helper T cell defect does not appear to be the sole cause since there was no IgA synthesis by the peripheral blood mononuclear cells of 9 of the 10 patients with a profoundly reduced serum IgA even when co-cultured with normal T cells. Furthermore, the cells of the nine patients with profoundly reduced IgA levels examined also failed to produce IgA when stimulated with the relatively helper T cell-independent polyclonal activators, *Nocardia* water soluble mitogen or Epstein-Barr virus. Taken together these data support the view that the reduced immunoglobulin synthesis of these patients is due to defects of both B cells and helper T cells. Such a broad defect in lymphocyte maturation taken in conjunction with our demonstration of persistent alpha fetoprotein production by ataxia telangiectasia patients provides support for the proposal that these patients exhibit a generalized defect in tissue differentiation.

INTRODUCTION

Ataxia telangiectasia (AT)¹ is an autosomal recessive, multisystem disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, recurrent sinopulmonary infections, a high incidence of neoplasia, and a variable immunodeficiency state (1-5). The patients have an increased sensitivity to ionizing radiation that may be associated with a defect in DNA repair (6-9). In addition, these patients have been shown to have an embryonic appearing thymus (4, 10) and the persistent production of the fetal protein of hepatic origin,

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¹ *Abbreviations used in this paper:* AT, ataxia telangiectasia; EBV, Epstein-Barr virus; NWSM, *Nocardia* water soluble mitogen; PWM, pokeweed mitogen.

alpha fetoprotein (11), features that support the hypothesis that they have a defect in tissue differentiation.

Recurrent infections are a major clinical feature in patients with AT (4, 5). They occur in >80% of the patients and may dominate the clinical picture leading to bronchiectasis and ultimately respiratory failure and death. The recurrent infections are associated with an immunodeficiency state affecting both cellular and humoral immune systems. In the case of cellular immunity, the patients, as a group, have impaired skin test responses to recall antigens and have delayed allograft rejection (4). The patients have modestly reduced lymphocyte numbers as well as reduced proportions of lymphocytes bearing T cell markers. In addition the *in vitro* proliferative responses to both mitogens and specific antigens is reduced in two-thirds of the patients. One of the most striking and consistent abnormalities associated with these disorders of the cellular system is the absence or an abnormality of the development of the thymus (4, 10). The thymus of AT patients does not show corticomedullary demarcation or Hassall's corpuscles and, thus, has the appearance of an immature or fetal thymus.

In the area of humoral immunity, the patients can make antibodies in response to challenge with a variety of bacterial antigens (both protein and polysaccharide) although these responses are impaired when compared with those of normals (2, 4, 12, 13). The response to viral antigen immunization and the antibody responses following infection with viruses provide more uniform patterns in that most patients manifest poor responses (4). The disorders of antibody responses are associated with abnormalities of immunoglobulin levels. 70% of the patients have an absence or an extreme deficiency of serum and secretory IgA (3, 4, 14) and 80% manifest IgE deficiency (4, 15, 16). The patients have a reduced serum concentration of the IgG₂ subclass (17). In addition, 80% of the patients have low-molecular weight (8S) IgM in their serum (4, 18).

Using metabolic turnover studies with radiolabeled immunoglobulins, we showed that the serum IgA and IgE deficiencies are due primarily to defects in the synthesis of these immunoglobulins (19). The present studies were directed toward gaining further insights into the pathogenesis of these disorders of immunoglobulin synthesis in patients with AT. We applied an *in vitro* biosynthesis approach to the study of the disordered immunoglobulin synthesis by the peripheral blood mononuclear cells (PBMC) of patients with AT. Most of these patients had diminished IgA production *in vitro*. Both intrinsic defects of the B cells that normally would mature into IgA-producing plasma cells as well as deficiencies of the helper T cells required for the terminal maturation of B cells appear to be

involved in the reduced IgA synthetic capacity observed in these patients.

METHODS

Patient population. 20 patients with AT undergoing evaluation at the National Cancer Institute were studied. All of these patients had cerebellar ataxia and oculocutaneous telangiectasia. In 14 cases they had an increased incidence of sinopulmonary infections as well. The serum IgG, IgA, and IgM concentrations shown were determined by radial diffusion in agar according to the method of Fahey and McKelvey (20). The IgA levels for patients with profoundly reduced serum IgA (i.e., <0.1 mg/ml) as well as the IgE and alpha fetoprotein concentrations were determined using double-antibody radioimmunoassay (RIA) techniques described previously (21). Each of the patients had an elevated alpha fetoprotein level.

Source of polyclonal activators. A series of materials were used in the *in vitro* studies to activate B lymphocytes to synthesize and secrete immunoglobulin molecules. Pokeweed mitogen (PWM) was obtained from Gibco Laboratories, Grand Island Biological Co., Grand Island, New York. *Nocardia opaca* water soluble mitogen (NWSM) was the kind gift of Dr. Constantine Bona and was prepared by a method previously described (22). Culture supernatants of the cell line B95-8 were used as infectious Epstein-Barr virus (EBV).

Measurement of immunoglobulin synthesis by lymphocytes *in vitro*. To study the transition of circulating B lymphocytes into immunoglobulin-secreting plasma cells, PBMC were cultured *in vitro* in the presence of PWM, a helper T cell-dependent polyclonal activator, NWSM, a relatively helper T cell-independent polyclonal activator, and the EBV, a helper T cell-independent polyclonal activator of B lymphocytes, using techniques we have previously described (23-25). The quantity of IgG, IgA, and IgM synthesized and secreted into the culture medium was then determined by double-antibody RIA, with techniques essentially identical to those previously described for IgE (21). Adequacy of the wash was confirmed for all cell populations by determining the immunoglobulin in the medium from 2×10^6 cells that had been incubated for only 30 min. These supernatants contained <50 ng of each class of immunoglobulins.

Preparation of T and B cell populations. Populations of normal B cells and monocytes freed of T cells were prepared by a two-step procedure that takes advantage of the observation that normal human T cells pass through anti-F(ab')₂ immunoabsorbent columns and form spontaneous rosettes with sheep erythrocytes, whereas normal B cells are selectively retained by these immunoabsorbent columns and do not form sheep erythrocyte rosettes. The technique of immunoabsorbent chromatography by Chess et al. (26) was modified (24) for the present study. The few residual T cells in the B cell fraction eluted from the immunoabsorbent column were removed by a sheep erythrocyte rosetting technique. The cells eluted from the column with gamma globulin were washed twice in RPMI 1640 medium containing 2 mM of L-glutamine and resuspended to 10×10^6 ml. 1 ml of the resuspended B cell fraction was added to 0.5 ml of the heat-inactivated fetal calf serum, previously absorbed with sheep erythrocytes, and 1.0 ml of a washed neuraminidase-treated sheep erythrocyte suspension containing 2% erythrocytes. The cells were mixed, incubated at 37°C for 10 min, then centrifuged at 800 rpm for 5 min, and stored at 4°C overnight. 16 h later the pellet was resuspended gently and the cells were layered over Ficoll-Hypaque and

centrifuged at 800 rpm for 10 min followed by 1,500 rpm for 25 min. The cells remaining at the interface were termed the "B cell" population. An additional fractionation to remove monocytes was not performed since these cells are essential for optimal immunoglobulin synthesis by B cells when cultured with PWM. The B cell population was then processed for *in vitro* immunoglobulin synthesis by washing as described above for unseparated lymphocytes. The T cells obtained by the anti-F(ab)₂ column separation technique discussed above contained <0.5% macrophages assessed by nonspecific esterase staining. However, these cells presumably contained sIg-negative, non-sheep erythrocyte rosetting cells. Therefore, T cell preparations obtained by the anti-F(ab)₂ column procedure were further purified by an overnight erythrocyte rosetting technique comparable to that utilized in the preparation of B cells. These T cell preparations did not synthesize immunoglobulins of any class when cultured with PWM *in vitro*.

Assays of helper cell activity. PWM-stimulated B cell populations rigorously freed of T cells do not synthesize immunoglobulin molecules (24, 27). The ability of added cells to augment immunoglobulin synthesis by these normal B cells was used as a measure of their helper cell activity (24, 25). The standard measures of helper activity consisted of quantitating the immunoglobulin synthesis of 5×10^5 normal B cells when co-cultured with 5×10^5 and in separate cultures with 5×10^4 irradiated cells from the individual to be studied. These values were compared with values for synthesis with identical numbers of the same normal B cells and irradiated normal T cells. The helper activity of patient and normal unirradiated T cells was also determined using comparable co-culture procedures. These T cell concentrations were chosen since, as shown in Fig. 1, the quantity of immunoglobulin synthesis observed at these concentrations is clearly sensitive to reductions in the number of T cells added. In further studies of the helper cell function of patients with AT the effect of the addition of irradiated normal T cells on the PWM-induced immunoglobulin synthesis by the patients' unseparated mononuclear cells was determined.

Assays of suppressor cell activity. PBMC from the patients with AT and from normal individuals were co-cultured (1×10^6 cells from each source) in 1 ml of medium in the presence of PWM (23, 25). The synthesis of immunoglobulins IgG, IgA, and IgM by cells of the two subjects in co-culture was related to the sum of the expected contribution by each cell population as follows: synthesis of Ig by the cells in co-culture as a percentage of the expected Ig = $100 \times$ synthesis of Ig by 10^6 cells from both subjects in co-culture \div by the sum of $\frac{1}{2}$ Ig synthesis by 2×10^6 cells from the patient and $\frac{1}{2}$ Ig synthesis by 2×10^6 cells of the control when cultured separately.

Assay of B cell activity. The function of the B cells of patients with AT was assessed by a number of procedures. In the first procedure the quantity of immunoglobulin synthesized by the mononuclear cells of these patients was determined when they were stimulated by the relatively helper T cell-independent activator NWSM, and by the helper T cell-independent activator EBV (25). In the second approach, the ability of the patients' unseparated mononuclear cells to synthesize immunoglobulin molecules when cultured with PWM and equal numbers of irradiated normal T cells was determined. Finally, the immunoglobulins synthesized and secreted by newly established EBV-induced B cell lines from patients and controls was determined. The EBV-transformed B cell lines were established by incubating T cell-depleted polymorphonuclear cell populations with culture supernatants of the cell line B95-8 to provide infectious EBV.

Statistical methods. The geometric mean values were used to estimate the average for IgG, IgA, and IgM produced by different cell populations. The \log_{10} of the immunoglobulin synthesized and secreted over the cultured period was used in all statistical tests.

RESULTS

The serum immunoglobulin levels of the 20 patients with AT studied are compared in Fig. 2 with the geo-

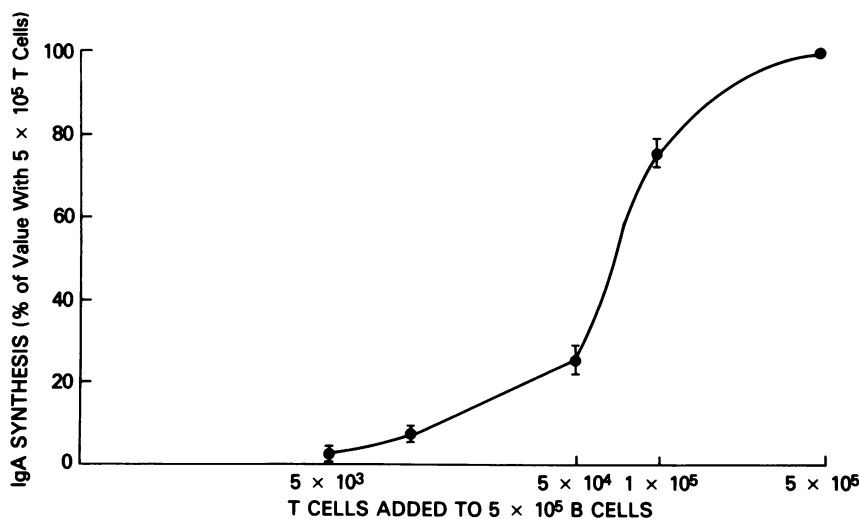


FIGURE 1 IgA synthesis by 5×10^5 PWM-stimulated purified B cells following addition of graded numbers of autologous irradiated (2,000 rad) T cells. On the basis of these observations helper activity of AT and allogenic cells was assessed by co-culture of 5×10^5 as well as 5×10^4 of these cells with PWM-stimulated B cells.

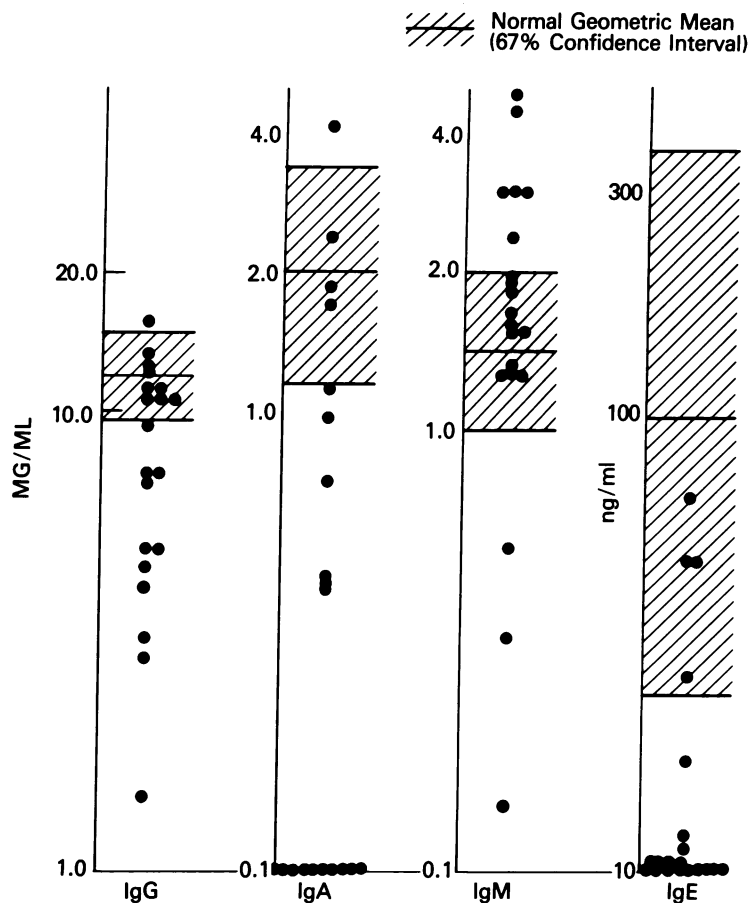


FIGURE 2 The serum immunoglobulin levels of 20 patients with AT are indicated by the dots and compared with values for normal controls. The geometric mean values and 67% confidence interval for normals on this and subsequent figures are indicated by the center line and the crosshatched area, respectively.

metric mean values and the 67% confidence intervals for serum IgG, IgA, and IgM concentrations for 20 normal individuals. The serum IgM levels were normal or slightly elevated in 17 cases and were slightly reduced in 3. Since the patients with AT frequently have monomeric (8S) IgM in addition to pentameric (19S) IgM in their circulation and the IgM standard used in radial diffusion is the pentameric species, the estimates presented for serum IgM for the patients using the diffusion in agar technique are frequently spuriously high. The serum IgG levels were within the 67% confidence interval for normal controls for 8 of the 20 patients, whereas the IgG concentrations for 11 of the patients were reduced below this level and in 3 cases were markedly diminished to 1.43–3.2 mg/ml. The serum IgE concentration was <15 ng/ml, the lower limit of the normal 90% confidence interval, in 15 of the 20 patients. No IgA was detectable in the serum of 10 of the patients using the diffusion in agar tech-

nique and in 3 others the IgA levels were below the lower 67% confidence interval limit of 0.68 mg/ml. In 5 of the 10 patients assayed with no detectable IgA by single diffusion in agar the serum IgA level was <100 ng/ml as assessed by double-antibody RIA, whereas in 2 patients, the IgA levels were 19,250 and 50,000 ng/ml.

The capacity of PBMC of the patients with AT as well as controls to synthesize IgG, IgA, and IgM was evaluated using the *in vitro* PWM-stimulated immunoglobulin biosynthesis system. The 17 normal controls studied synthesized geometric means (with 67% confidence intervals in parentheses) of 3,147 ng (2.9)² for

² The geometric mean is presented with 1 SD of the log of the geometric mean given in parentheses. The upper and lower limits of the 67% confidence interval were obtained by multiplying and dividing the geometric mean by this number, respectively.

IgG, 2,289 ng (2.54) for IgA and 3,676 ng (2.57) for IgM per 2×10^6 cells in culture for 12 d. There was a broad range in the values for immunoglobulin biosynthesis by the cells of the different AT patients that roughly paralleled their serum immunoglobulin levels (Fig. 3). The PBMC from the 17 AT patients examined had a geometric mean synthesis for IgM synthesis of 1,548 ng/ml. In 6 of these 17 cases < 550 ng of IgM, the lower limit of the normal 95% confidence interval, was synthesized during the culture period. The IgG synthesis of nine of the patients was within the normal 95% confidence interval, whereas the IgG synthesis of the remaining eight patients was markedly reduced to < 250 ng/ 2×10^6 cells in the PWM-stimulated cul-

tures. These eight patients with reduced IgG synthesis in vitro included the three patients with a serum IgG level < 3.2 mg/ml. The IgA synthesis by PWM-stimulated PBMC was < 100 ng for 9 of the 10 patients with profoundly reduced serum IgA as assessed by radial immunodiffusion. The remaining patient with 19,000 ng/ml of IgA in the serum detected by RIA synthesized 140 ng of IgA in vitro, a value below the lower limit of the normal 95% confidence interval. Five of the seven patients studied with serum IgA levels > 0.1 mg/ml synthesized IgA in vitro, in three cases within the normal 95% confidence interval. Thus, the synthesis of immunoglobulin by PWM-stimulated lymphocytes from patients with AT was reduced below

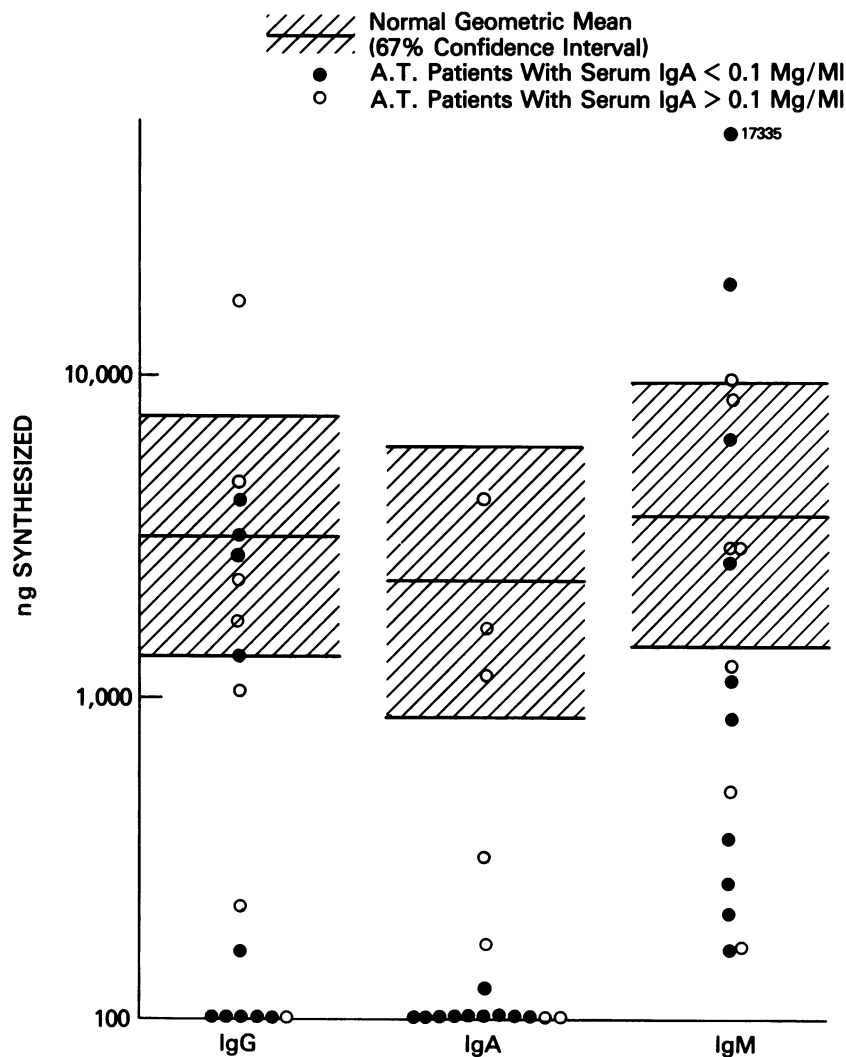


FIGURE 3 Immunoglobulin synthesis by PWM-stimulated unseparated PBMC from patients with AT. Values for patients with serum IgA levels < 0.1 mg/ml are indicated by solid dots whereas those with IgA levels > 0.1 mg/ml are indicated by open circles.

the normal 95% confidence interval in 6 cases for IgM and in 9 cases for IgG and in 14 cases for IgA including all of the cases with a serum IgA < 0.1 mg/ml.

To determine whether the reduced in vitro immunoglobulin synthesis by the patients was due to abnormal suppressor cell activity the circulating mononuclear cells from the patients were co-cultured with PBMC from normal subjects. The secretion of immunoglobulin by the PWM-stimulated cells from the two individuals in co-culture was related to the sum of the expected contribution of each cell population. The mean values for production of IgM, IgG, and IgA by 10 co-cultures of allogeneic lymphocytes from pairs of normal individuals ranged from 95 to 106% of the expected values. The cells of the 16 patients examined for excessive suppressor cell activity were co-cultured in separate studies with the cells from one to six normal individuals for a total of 49 co-culture studies between patient and normal cells. The geometric mean values for the immunoglobulin synthesis as a fraction of the expected value in these co-culture studies were 1.5 for IgG, 1.3 for IgA, and 1.2 for IgM. 2 of the 18 patients showed modest (50–80%) suppression of all classes of immunoglobulin when co-cultured with normal lymphocytes. However, none of the patients showed profound suppression, that is >80% suppression of all classes and no patient showed class-specific suppressor cell activity in these co-culture studies. Thus, the reduced serum immunoglobulin concentrations and reduced in vitro immunoglobulin biosynthetic capacity of the PBMC of patients with AT does not appear to be due to excessive suppressor cell activity.

As noted above, patients with AT have an immature appearing thymus associated with an array of disorders of T cell-mediated immunity. Therefore, a series of approaches was used in the present study to define whether a defect of helper T cell activity contributed to the reduced immunoglobulin synthesis by AT patients. As noted above, PWM-stimulated B cells, rigorously freed of T cells, do not secrete immunoglobulins. The ability of added irradiated cells from patients with AT to augment immunoglobulin synthesis by such normal purified B cells was used as a test for the helper activity of the patients' cells. The helper activity of the cells from patients with AT was compared to the helper activity provided by equal numbers of irradiated T cells from the individual who was the B cell source as well as that provided by normal irradiated cells that were allogeneic to the indicator B cells. When 5×10^5 normal B cells were co-cultured with equal numbers of irradiated cells from six patients with AT, the AT cells provided help for IgG, IgA, and IgM synthesis that was 48 (2.0), 20 (1.9), and 16 (2.1) %, respectively, of that provided by equal numbers of autologous irradiated cells. Patients also had depressed

helper cell activity when co-cultured at a ratio of 5×10^4 patients' irradiated cells/ 10^5 normal B cells, providing only 25, 21, and 19% of the helper activity provided by autologous irradiated T cells for IgG, IgA, and IgM synthesis, respectively (Fig. 4). In contrast, normal allogeneic irradiated cells provided 75 (1.23), 82 (1.25), and 71 (1.14) % of the help for IgG, IgA, and IgM provided by autologous irradiated T cells. Thus, the help provided by the patients' cells was less than that provided by normal allogeneic cells. Since, as noted above, the cells of patients with AT show a greater sensitivity to X-radiation than do normal cells, the helper studies were repeated comparing the help provided by unirradiated cells from patients with AT to that provided by unirradiated autologous T cells. Again, the cells of the patients with AT had reduced helper cell capacity that was, on a mean, only 17% of that of the autologous normal T cells. It should be noted that although the helper T cell capacity of patients with AT was significantly reduced, the cells of all patients manifested at least some helper cell capacity.

To define the contribution of the helper T cell deficiency to the disorder of immunoglobulin synthesis in these patients more fully, the effect of adding normal irradiated T cells to the PWM-stimulated unseparated mononuclear cells from AT patients was determined (Fig. 5). There was an augmentation of IgM synthesis by the AT mononuclear cells following the addition of irradiated normal T cells for 15 of the 17 patients studied. In addition, IgG synthesis increased following the addition of normal irradiated T cells for 9 of the 12 patients studied who had been able to synthesize at least 100 ng of IgG before the addition of T cells. However, the addition of normal T cells did not lead to augmentation of IgG synthesis when added to the cultures of mononuclear cells from the five patients who had not been able to synthesize any IgG when cultured alone. There was an augmentation of IgA synthesis following addition of irradiated normal T cells to the cultures of the cells of each of the patients who had a serum IgA concentration > 0.1 mg/ml. In contrast, no IgA synthesis was observed by the PWM-stimulated mononuclear cells of eight out of the nine patients with a serum IgA concentration < 0.1 mg/ml even following the addition of irradiated normal T cells to the cultures. In control studies there was a modest augmentation of immunoglobulin synthesis when normal irradiated T cells were added to PWM-stimulated unseparated mononuclear cells of normal individuals, in some cases with ratios of synthesis in the presence of added T cells to that in the absence of added T cells of 0.98 (1.6) for IgG, 3.0 (1.33) for IgA, and 1.99 (1.43) for IgM. However, the increases in these cases were less than those that were observed

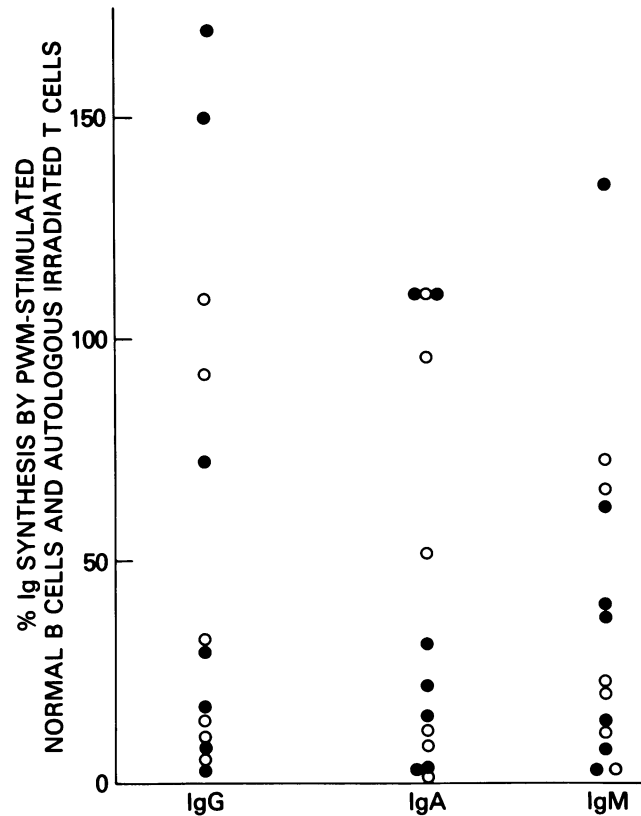


FIGURE 4 Helper activity for 5×10^5 PWM-stimulated purified normal B cells provided by 5×10^4 irradiated (2,000 rad) AT cells. The values for the patients' cells are expressed as a percentage of the help provided by equal numbers of autologous irradiated T cells that were studied simultaneously. Values for patients with serum IgA levels < 0.1 mg/ml are indicated by solid dots whereas those with IgA levels > 0.1 mg/ml are indicated by open circles.

following addition of irradiated T cells to the cultured cells of those AT patients who had been able to synthesize these immunoglobulin classes in vitro before the addition of normal T cells. The observation that patients with AT had diminished helper T cell activity when compared with normal individuals and that the addition of normal T cells augmented immunoglobulin synthesis by AT cells suggests that diminished helper T cell activity contributes to the diminished immunoglobulin synthesis in some patients.

However, a number of the observations discussed above suggest that a deficiency of helper T cells is not the sole cause of the disordered immunoglobulin synthesis of AT patients. Some helper cell activity was demonstrable in the cell populations from each of the patients as shown in co-cultures of the patients' irradiated cells and normal B cells, and yet 10 of the patients synthesized no IgA. In addition, the PBMC of 9 of the 10 patients who had a serum IgA of < 0.1 ng/ml did not synthesize IgA even when co-cultured

with normal T cells. Furthermore, the mononuclear cells of these patients did not synthesize IgA when co-cultured with irradiated (2,000 rad), unseparated mononuclear cells from normal individuals suggesting that a macrophage defect was not the cause of this observed defect in immunoglobulin synthesis. To analyze the B cell function of AT patients further, we cultured these mononuclear cells with relatively helper T cell-independent activators of B cells including NWSM (Fig. 6). Normal unseparated mononuclear cells had geometric means for synthesis of 1,388 ng for IgG, 909 ng for IgA, and 2,110 ng for IgM/ 2×10^6 mononuclear cells when cultured for 12 d with NWSM. Rigorously T cell-depleted normal B cells stimulated with NWSM synthesized all three immunoglobulin classes. However, NWSM appears to be only a relatively T helper cell-independent activator, since the addition of normal T cells to these B cells in culture lead to augmented immunoglobulin synthesis (25). The geometric means for immunoglobulin syn-

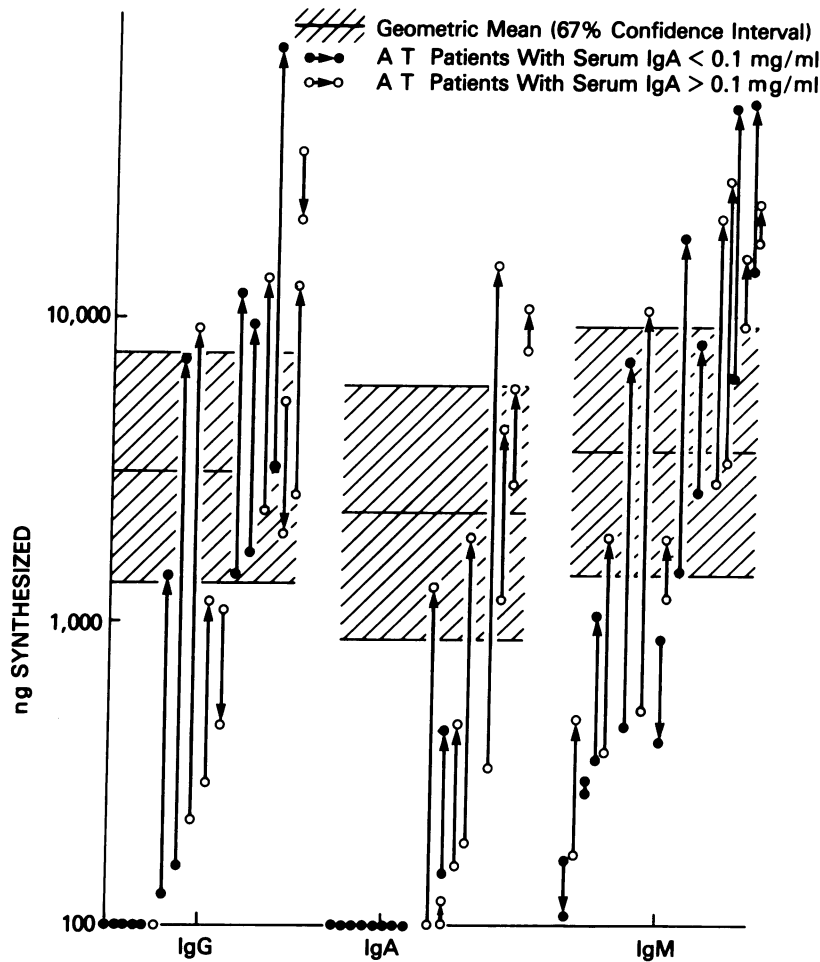


FIGURE 5 The effect of co-culturing normal irradiated T cells with AT cells on the immunoglobulin synthesis by PWM-stimulated AT mononuclear cells. Immunoglobulin synthesis by the mononuclear cells of particular patients before and after addition of normal irradiated T cells are connected by arrow with the arrow pointing to the value for immunoglobulin synthesis after addition of normal T cells. Single dots at the base of the chart without connecting arrows represent patients who did not synthesize significant amounts of the isotype indicated before or after addition of normal T cells. Solid dots represent patients with serum IgA levels < 0.1 mg/ml, whereas open circles represent values for patients with serum IgA levels > 0.1 mg/ml.

thesis of unseparated mononuclear cells from the patients with AT stimulated by NWSM were 512 ng for IgG, 122 ng for IgA, and 1,984 ng for IgM. However, none of the nine patients examined with a serum IgA concentration of < 0.1 mg/ml synthesized any IgA in the presence of this activator. The EBV is also an excellent activator of human B cells. Normal mononuclear cells stimulated with EBV had geometric means for immunoglobulin synthesis of 279 ng of IgG, 764 ng of IgA, and 598 ng of IgM/ 2×10^6 cells in culture (Fig. 7). With this activator, the immunoglobulin synthesis by 1×10^6 T cell-depleted B cells from six normal individuals was 424 ng for IgG, 745 ng for IgA, and 2,093 ng for IgM. The addition of autologous T

cells to these normal B cells did not augment or depress their immunoglobulin synthesis with geometric mean values for IgG, IgA, and IgM of 251, 516, and 2,736 ng, respectively. Thus, in the present system, EBV is a helper T cell-independent polyclonal activator of human peripheral blood B cells. The unseparated mononuclear cells of the patients with AT synthesized 213 ng of IgG, 130 ng of IgA, and 1,076 ng of IgM when stimulated by the EBV. In accord with the data obtained with NWSM, none of the nine AT patients examined who had a serum IgA concentration of less than 0.1 mg/ml synthesized IgA in vitro when stimulated by the EBV. To obtain additional information concerning the capacity of the B cells of AT patients

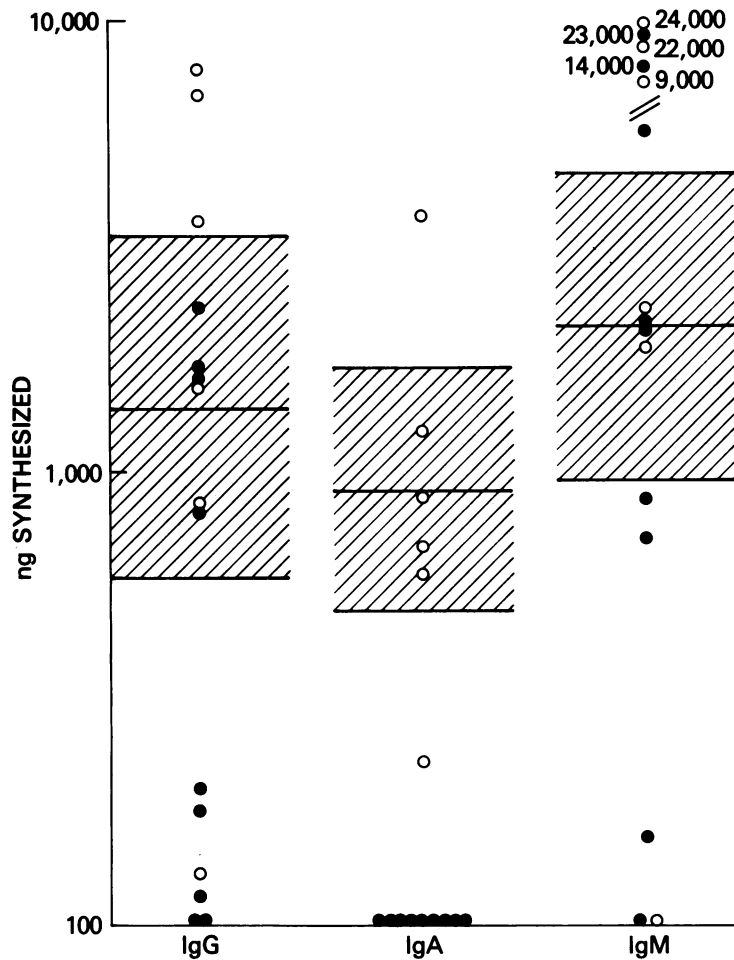


FIGURE 6 Immunoglobulin synthesis by AT PBMC stimulated by NWSM.

to synthesize different immunoglobulin isotypes we defined the immunoglobulins synthesized and secreted by four newly established (i.e., <3-mo-old) cultures of EBV-induced B cell lines from two patients with AT and IgA deficiency and compared this to the pattern of immunoglobulin synthesis by comparable cultures from four normal individuals. Each of the newly established B lines from normal individuals synthesized all three major isotypes, IgG, IgA, and IgM, whereas the comparable cultures from the patients with AT and IgA deficiency synthesized IgM and IgG but not IgA. The observations presented support the view that a B cell maturation defect plays a role in the pathogenesis of the disorders of IgA synthesis observed in patients with AT.

DISCUSSION

Patients with AT have a series of disorders of humoral immunity that are frequently associated with abnor-

malities of the levels of immunoglobulin in the serum and external secretions. The majority of patients have an absence or marked deficiency of serum IgA, IgE, and the IgG₂ subclass, and frequently produce low-molecular weight IgM (3, 4, 14-18). In this study we show that these in vivo abnormalities of immunoglobulin levels are paralleled by a reduced capacity for in vitro immunoglobulin biosynthesis by polyclonally activated PBMC from patients with this disorder. The amount of PWM-induced immunoglobulin synthesis was below the lower limit of the 95% confidence interval for IgM for 6, for IgG for 8, and for IgA for 14 of the 17 patients studied. A similar deficiency of lymphocyte immunoglobulin synthesis has been demonstrated to be associated with excessive activity of suppressor T cells in certain patients with other primary immunodeficiency disorders such as common variable immunodeficiency (23) and thymoma and hypogammaglobulinemia (28). In addition, IgA class-specific

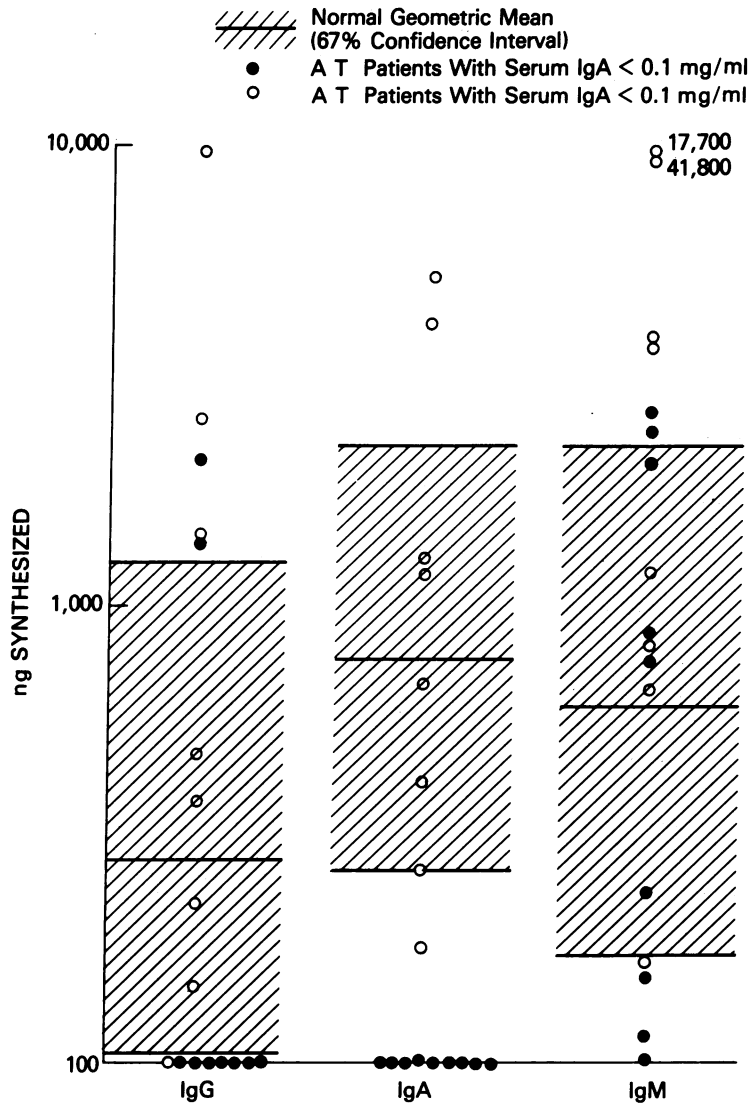


FIGURE 7 Immunoglobulin synthesis by AT PBMC stimulated by EBV.

suppressor T cells that inhibit the maturation of IgA-bearing B cells into IgA-secreting plasma cells have been demonstrated in a subset of patients with selective IgA deficiency (29). No evidence for such excessive suppressor cell activity affecting all immunoglobulins or affecting the IgA class alone was obtained for the AT patients examined.

The patients with AT did have deficiencies of helper T cell function as assessed by a modification of the in vitro biosynthesis technique in which subpopulations of cells from the patients and from normals were cocultured in the presence of PWM. This approach takes advantage of the fact that PWM stimulation of B cells is absolutely dependent on the presence of helper T

cells. The basic assumption underlying this approach is that allogeneic T cells can help B cells to differentiate into immunoglobulin-producing cells. This assumption appears to be fulfilled for PWM since it has been shown that in the PWM system the allogeneic normal T cells (and their soluble products) provide help to normal B cells to essentially the same degree as do autologous T cells (25, 30, 31). The patients with AT fulfill the two required criteria we outlined previously (25) for the conclusion that diminished helper cell activity contributes to the immunoglobulin deficiency of these patients; that is, the cells of all of the patients had reduced (but measurable) helper T cell activity for immunoglobulin synthesis by co-cultured

normal B cells with a geometric mean helper activity that was 22% of normal. Furthermore, the addition of normal irradiated T cells to the patients' PBMC led to an augmentation of IgM synthesis in 15 of the 17 patients studied and to an increased IgG and IgA synthesis by the majority of the cell populations from those patients who had synthesized these isotypes *in vitro* before the addition of the normal T cells to the cultures. These observations using the *in vitro* PWM-stimulated immunoglobulin biosynthesis system paralleled our observations with an antigen-specific antibody production system in which we showed that the antibody response to sheep erythrocytes *in vitro* was diminished in patients with AT due in part to a deficiency of T helper cells (32). Furthermore, the functional deficiency of helper T cells was paralleled by a relative deficiency of T cell subsets that bear surface markers associated with helper inducer functions. Trumpeter et al. (33) determined that there was a reduced proportion of T cells that had receptors for IgM. The patients in our study had a similar deficiency of the T_H subset. Furthermore, the proportion of the T cells of the patients that reacted with the monoclonal antibody T4 that identifies helper/inducer cells (34) as well as some suppressor cells was reduced with an arithmetic mean value for our patients studied of 30%±5 as compared with the mean of normal individuals of 50±1.8. In addition, the mean of the ratios of T cells reactive with T4 monoclonal antisera to those reactive with the T8 monoclonal was reduced to 0.78±0.27 in the patients as compared with the mean of 1.69±0.14 in normal controls.

As noted above, a number of observations suggest that an intrinsic defect of B cells also plays a major role in the deficient synthetic capacity of AT mononuclear cells. First, mononuclear cells of the patients with a serum IgA of < 0.1 mg/ml do not secrete IgA into the media even when stimulated by relatively T helper cell-independent polyclonal activators such as the EBV and NWSM. In addition, in 9 of the 10 cases the cells of these patients did not synthesize IgA *in vitro* when their PWM-stimulated cells were co-cultured with normal T cells that provide helper activity. Finally, newly established EBV-transformed B cell lines from two patients studied with AT did not synthesize IgA in contrast to cell lines from normal individuals. Thus, the humoral immunodeficiency of patients with AT appears to be due to both a defect in the terminal maturation of B cells into IgA- and IgE-producing plasma cells due to an intrinsic defect of these B cells as well as to a disorder of helper T cell activity that is required for this B cell maturation.

The underlying defect in patients with AT has not been defined as yet. Nevertheless, certain important clues have emerged which suggest two predominant

hypotheses concerning the pathogenesis of this disease. One hypothesis is based on the fact that patients with AT have increased sensitivity to ionizing radiation (6-9). Cultured AT fibroblasts have an abnormal X-ray sensitivity that parallels the hypersensitivity to clinical radiotherapy *in vivo*. This observation suggested that AT cells might be deficient in repair of radiation damage perhaps as a result of a DNA repair defect. In this regard, in contrast to normal cells that tend to inhibit DNA synthesis after irradiation, presumably to allow more time for damage to be repaired, AT cells do not do so and the damage appears to be fixed during DNA replication and the cells die (35). There have been many attempts to show a DNA repair defect in AT biochemically. However, so far all tests for single or double stranded break repair have failed to show any convincing deficiency (36). In addition to observations concerning abnormal X-ray sensitivity, there have been studies indicating chromosomal aberrations in patients with AT. These patients have a marked increase in the incidence of chromosomal translocations especially involving chromosome 14 (7, 37-41). The deleted portion of this chromosome has been found to be translocated to the other chromosome 14, the X chromosome, or to chromosome 7. In light of the frequent translocations and breaks of chromosome 14 found in patients with AT, it is of interest to note that the genes coding for the human immunoglobulin heavy chains including IgA and IgE are on chromosome 14 (42). Recently, an understanding of the immunoglobulin genes and the rearrangements that they undergo as a stem cell matures into an IgA- and IgE-producing plasma cell has emerged from a number of laboratories (43-47). The immunoglobulin heavy chain genes are comprised of discontinuous gene segments which code for the subdivisions of the variable region and others which code for the separate constant (C) regions of C_μ, C_δ, C_γ, C_ε, and C_α. This is an extraordinarily dynamic region of DNA that within developing B lymphocytes undergoes rearrangements that essentially move these gene segments within the genomic DNA. The first DNA rearrangement event is the assembly of an intact variable region gene from its subsegments after which this recombined V_H gene is then transcribed together with the first constant region (C_μ) to produce IgM. When a cell switches from production of IgM to the IgG or IgA isotype, it undergoes a second distinctive form of rearrangement that deletes intervening DNA between the assembled variable region and the expressed C_γ or C_α region. This deletional recombination occurs within specialized switch areas located on the 5' side of each constant region and are comprised of highly homologous stretches of short repetitive units that appear to facilitate this second recombinational event. Since patients

with AT frequently have translocations and breaks of chromosome 14 and since many of the patients in this study appear to have intrinsic B cells defects associated with failure of IgA production, radiolabeled DNA probes to the constant alpha region genes were applied to the study of the immunoglobulin A genes in cells from five of the AT patients with undetectable serum IgA (i.e. <100 ng/ml). Using this probe, each of the patients examined had retained the constant alpha (C_α) genes indicating that an absence of a constant alpha gene was not the cause of the failure of IgA production by these patients. However, a disorder of the molecular events on this chromosome, such as an abnormality of the switch from C_μ to C_α genes, is still a possibility. That is, it is possible that a deficiency of a factor needed for recombination and repair of DNA could account for both the failure of the switch from IgM to IgA and IgE production as well as the radiosensitivity and chromosomal instability observed with AT patients.

In an alternative hypothesis, it was suggested that there is a defect in tissue differentiation in patients with AT (10, 11). The first observation that suggested this possibility was the fact that patients with AT have an abnormal development of their thymus and have a thymus that is not atrophic but that has the appearance of a fetal thymus (10). In this regard it had been shown by Auerbach (48) that thymic development is dependent on the interaction between entodermally derived epithelial elements and mesenchymally derived lymphoid elements. Peterson et al. (10) proposed that a defective interaction between these two major germ lines, the entoderm and mesoderm, might explain the failure of thymic maturation in patients with AT. A consequence of such a postulated disorder would be a failure in the development and maturation of lymphoid tissues as well as other tissues that involve an interaction between the primitive mesenchyme and entoderm. To examine this hypothesis further, we have determined the serum alpha fetoprotein level in patients with AT (11). Alpha fetoprotein is a protein produced by the fetal liver that is normally present in high concentration in the fetus, but in an exceedingly low concentration (<20 ng/ml) in the serum of normal individuals >6 mo of age. In our initial observations (11), we found that all 20 patients with AT examined had an elevated alpha fetoprotein level with a range from 44 to 2,800 ng/ml. In contrast, none of the siblings or parents of patients with AT or patients with various other immunodeficiency or neurological disorders had elevated alpha fetoprotein levels. Subsequently we have extended our original series and have demonstrated that 59 of 60 patients with AT we examined had an elevated serum alpha fetoprotein level. Our observations on alpha fetoprotein have been confirmed (49). Furthermore, it has been noted that pa-

tients with AT have an elevated level of a beta fetoprotein (50) and in some cases of carcinoembryonic antigen as well (49). The view that AT is associated with tissue immaturity is supported by the observation that these patients frequently have ovarian agenesis (3-5).

As far as the immune system is concerned an important consequence of the postulated abnormality of tissue maturation is the failure of normal thymic development. This failure could explain the abnormalities of the T cell limb of the immunologic pathway including the abnormalities of delayed hypersensitivity, of skin graft rejection, as well as the abnormalities of T cell-mediated cytotoxicity (4, 5, 10, 13). The helper T cell abnormalities demonstrated in the present *in vitro* immunoglobulin biosynthesis studies could also be a consequence of the failure of normal thymic development. As noted above, the deficiency of IgA and IgE production may be related in part to this deficiency of helper T cell activity. However, in addition, an intrinsic defect of B cell maturation was demonstrated that contributes to these deficiencies as well. The disorder in the terminal maturation of B cells that would normally develop into IgA- and IgE-producing plasma cells might be secondary to the suggested defect in tissue maturation and mesenchymal/entodermal interactions considered above. This is especially true since IgA- and IgE-producing cells frequently underlie the gastrointestinal and respiratory tracts and might require microenvironments in these sites that are ultimately dependent on such germ line interactions.

In summary, it appears that the X-ray sensitivity and the potential DNA repair defect are most capable of explaining the various degenerative changes in AT including the central nervous system changes and premature senescence whereas the abnormalities of tissue differentiation are most capable of explaining the failure of organ maturation and the immunological abnormalities. Clearly, these two hypotheses are not mutually exclusive and a unitary hypothesis will have to be developed. One possibility suggested by analogy with the *recA* mutation in bacteria (36) is that common enzymatic steps exist that are important in some types of DNA repair and for some types of cellular maturation especially those involving recombinational events such as those involved in the activation of immunoglobulin genes. A failure in such an enzyme system would, thus, lead to both increased X-ray sensitivity and to a failure in the normal maturation of certain tissues that are critical for the generation of immunoglobulin molecules.

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