calculated separately for four centers with large numbers of subjects and for the other seven centers. In all cases, the older women had lower success rates (Table 2).

DISCUSSION

This study has shown that a decrease in fecundability (conception rate per cycle) as a function of a woman's age is slight but significant after 30 years of age and marked after 35 years. The probability of success of AID for 12 cycles, which was 73 per cent and 74 per cent for the two groups of women under 31, dropped to 61 per cent for those 31 to 35 and to 54 per cent for those over 35. The large decrease in this last group was not simply due to the inclusion of women over 40, since the subgroup that was 36 to 40 had the same low probability of success. The decrease in fecundability with age is consistent across the CECOS centers (Table 2) and supports the reliability of the findings.

In any attempt to study variations in fecundity as a function of a woman's age, two major problems are encountered. The first is the need to separate the influence of the age of the woman from associated variables such as the pattern of coitus and the age of the husband. In our study it was possible to control these variables through the use of artificial insemination with frozen semen. Furthermore, the characteristics of the donors and the insemination cycles were similar in all age groups.

The second problem is that the variable under study — the age of the woman — can itself result in bias, since time introduces a type of selection. In AID this possibility is especially high if a husband has reduced fecundity but is not sterile; if his wife is very fecund, she may be precluded from study because she has previously conceived. This bias becomes more pronounced with the age of the women studied. This is the reason for our choosing to study only women with azoospermic husbands. It is indeed possible that the choice of population could have introduced bias because of factors such as previous marriages, previous attempts at conception by AID, or adultery. In our study, the proportions of remarried women and of those who had already been inseminated were very low (<1 per cent). It is impossible to evaluate the incidence of adultery, but it is unlikely that adultery accounted for the findings of this study.

Table 1. Rates for Success, Loss to Follow-up, and Dropping Out, According to Age Group.

RATE	PERCENTAGE			
	≤25 yr	26–30 yr	31–35 yr	>35 yr (36–40)
Mean rate per cycle				
Successes	11.0	10.5	9.1	6.5 (6.5)
Losses to follow-up	2.8	2.5	2.4	2.4
Dropouts	4.0	4.0	4.7	4.9
Cumulative success rate after 12 cycles	73.0	74.1	61.5	53.6 (55.8)

Table 2. Mean Success Rates at CECOS Centers.

AGE GROUP	PER CENT OF SUCCESSES PER CYCLE *				
	CENTER 1 (2701)	CENTER 2 (1854)	CENTER 3 (1789)	CENTER 4 (779)	CENTER 5–11 (2877)
≤35 yr	10.9	12.6	7.7	12.6	8.9
>35 yr	6.5	7.9	5.6	6.8	5.4

*Figures in parentheses denote number of cycles.

The many variables encountered in attempts to study the effect of age on female fecundity make it necessary to find various approaches to this problem. One such approach is through artificial insemination with frozen semen, as in this study. It is difficult to know to what extent our results approximate those of natural reproduction, but artificial insemination with frozen donor semen now appears to provide the best means of reducing the influences of associated variables and sources of bias to a minimum.

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TRANSIENT ANTIBODY DEFICIENCY AND ABNORMAL T SUPPRESSOR CELLS INDUCED BY PHENYTOIN

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THE lack of normal B-cell differentiation in patients with acquired or congenital antibody deficiency may reflect abnormalities inherent in cells of B-lymphoid lineage or may involve abnormalities of regulatory (suppressor) cells.[1,2] Circulating suppressor T cells, which interfere with IgM secretion in a plaque-forming-cell assay, have been found in 60 per cent of these patients, but their role in the pathogenesis of the disease is unclear.[3,4]

We observed the development of abnormal suppressor T cells and hypogammaglobulinemia in a

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patient receiving phenytoin (diphenylhydantoin). After the drug was withdrawn, the suppressor activity disappeared and immunoglobulin production resumed. This transient, drug-dependent expression of both suppressor-cell activity and antibody deficiency strengthens the argument that both events may be causally related.

CASE REPORT

A healthy, well-developed 11-year-old boy was given phenytoin for post-traumatic seizures. After 12 months of therapy, he had striking, generalized edema, a confluent, desquamating erythematous rash over the entire body, and generalized lymphadenopathy with tender firm nodes. The liver and spleen were somewhat enlarged, the temperature fluctuated around 39°C, both ears were draining, and the throat was diffusely erythematous; coughing produced some greenish sputum. The drug was stopped, and hydroxyzine hydrochloride (Atarax, 2 mg per kilogram of body weight per day) was given for six days. The edema, rash, and lymphadenopathy began to recede promptly and were completely resolved at discharge four weeks later.

METHODS

Cell Preparations

All procedures for preparing the cells have been described in detail previously.[3] In brief, peripheral-blood mononuclear cells were obtained by separation on Ficoll–Hypaque gradients. When indicated, T lymphocytes were purified by E-rosette depletion. Enriched theophylline-resistant and theophylline-sensitive T-lymphocyte subpopulations were obtained in a second E-rosette depletion in the presence of theophylline (3 mM) as described elsewhere.[4]

Tissue Culture and Plaque-Forming-Cell Assay

For ovalbumin-specific plaque-forming-cell responses, 3×10^6 cells were incubated at 37°C in 10 ml of supplemented RPMI-1640 culture medium with 0.01 to 100 μg of ovalbumin (Sigma Chemical, St. Louis, Mo.).[5] After five to seven days of culture, direct (IgM) ovalbumin-specific plaque-forming-cell responses were measured with the use of poly-L-lysine-coupled monolayers of ovalbumin-coated erythrocytes and guinea-pig complement.[6] To detect spontaneous suppressor-cell activity, various quantities of cells from blood freshly drawn from the patient and a control were mixed with a constant number of previously generated plaque-forming cells. After 60 minutes of incubation at 37°C, the residual plaque-forming activity of these mixtures was determined in triplicate.[4]

RESULTS

Relevant laboratory findings are shown in Table 1. On admission there was leukocytosis, with 25 per cent atypical lymphocytes, eosinophilia, and evidence of antibody deficiency. Despite immunization with two booster doses of tetanus, diphtheria, and poliomyelitis antigens, there was no rise in antibody titers. A lymph-node biopsy showed interfollicular hyperplasia and a reduction in the number and size of the primary and secondary follicles. Plasma cells were absent in bone marrow and lymph nodes. The bone marrow, lymph node, and two blood samples were negative for Epstein–Barr virus, according to immunofluorescence testing and tissue-culture analysis (kindly performed at the Department of Infectious Diseases, Yale University, New Haven, Conn.). The results of serology and studies of the urine were negative for cytomegalovirus.

Table 1. Laboratory Evaluation of the Patient.

FINDINGS	VALUE		
	ON ADMISSION	AFTER 5 WK	AFTER 10 WK
Hematologic			
White cells ($/\mu l\times10^{-3}$)	28.4	13.3	9.5
Granulocytes (per cent)	25	44	44
Eosinophils (per cent)	20	8	4
Lymphocytes (per cent)	25	48	41
Atypical lymphocytes (per cent)	25	0	0
Immunologic			
Schick test	Positive	Not done	Negative
Isohemagglutinins (titer)	1:2–1:4	1:8	1:32
IgG (mg/dl)	220	350	500
IgM (mg/dl)	<10	30	70
IgA (mg/dl)	<10	20	40
IgE (ng/ml)	<10	Not done	165

Lymphocyte Studies

Although a normal proportion of peripheral-blood lymphocytes formed E-rosettes, the number of B lymphocytes bearing surface immunoglobulin was profoundly decreased. The patient's cells functioned normally when stimulated with T-cell mitogens or allogeneic cells (Table 2).

The cells generated a low but statistically significant anti-ovalbumin plaque-forming-cell response ($P<0.001$) (Table 2). However, when such cultures were stimulated with the antigen combined with pokeweed mitogen, a response was no longer observed, whereas cells from normal donors generated somewhat higher numbers of plaque-forming cells in the presence of antigen plus mitogen. These findings resembled those obtained in patients with antibody deficiency. As discussed previously,[4] the effects of pokeweed mitogen in abrogating rather than enhancing plaque-forming-cell responses suggested the presence of an abnormal, mitogen-induced suppressor-cell function in this patient.[1,4,6] Lymphocytes from donors who were receiving phenytoin but had normal serum immunoglobulin levels had normal responses and were not different from those of the normal controls.

Spontaneous Suppressor Activity

To detect spontaneous suppressor-cell activity, 2.1×10^4 cultured cells containing 60 plaque-forming cells specific for ovalbumin were mixed with equal numbers of fresh mononuclear cells or purified lymphocyte subpopulations obtained from the patient and a normal control (Fig. 1). The residual plaque-forming-cell responses were assayed after 60 minutes of incubation at 37°C. Plaque formation was suppressed by only the patient's cells, in particular the theophylline-sensitive T-cell subset. Spontaneous suppressor activity did not occur in fresh lymphocytes from over 100 normal donors[4] and from several patients who were receiving phenytoin but who had normal immunoglobulin levels.

Spontaneous suppressor-cell function in patients with humoral immune deficiency may involve the ac-

Table 2. Lymphocyte Distribution and Function in the Patient and Controls.

FEATURE	VALUE		
	ON ADMISSION	AFTER 5 WK	AFTER 10 WK
Cell markers			
B lymphocytes (per cent sIg^+) *	<0.1	0.4	2.5
T lymphocytes (per cent E rosettes)	58	49	56
Lymphocyte proliferation ($cpm\times 10^{-3}$) †			
Phytohemagglutinin	47±2	53±4	57±3
Concanavalin A	39±4	37±3	35±3
Pokeweed mitogen	12±2	16±2	15±1
Mixed lymphocyte reaction			
Response	64±5	58±6	69±8
Stimulation	76±7	65±4	81±9
PFC response (per culture) ‡			
To ovalbumin			
Patient	510±40	880±110	1730±120
Normal controls	1950±140	1790±120	1860±90
Controls given phenytoin	1630±100	1810±90	1480±130
To ovalbumin plus pokeweed mitogen			
Patient	<200	320±60	2200±40
Normal controls	2360±110	2510±190	2490±170
Controls given phenytoin	2100±180	2660±170	2030±220

*sIg^+ denotes cells that are surface-immunoglobulin positive.

†Mean ±1 S.D. counts per minute of [^{3}H]thymidine incorporation in cultures of 5×10^4 responding cells.

‡Mean ±1 S.D. number of ovalbumin-specific, direct hemolytic plaque-forming cells (PFC) generated per culture of 3×10^6 peripheral-blood lymphocytes in the presence of 0.3 μg of ovalbumin. Controls given phenytoin had normal levels of immunoglobulin.

tivation of adenylate cyclase and elevated cyclic AMP levels.[4,7,8] To characterize the suppressor-cell activity in the patient described above, lithium (a blocker of adenylate cyclase activation), salbutamol (a β-agonist), and dibutyryl cyclic AMP were added to the suppressor assay. As shown in Figure 1, lithium prevented the expression of suppressor-cell activity, but salbutamol and dibutyryl cyclic AMP enhanced it. Furthermore, lithium interfered with the suppression induced by salbutamol but not that induced by dibutyryl cyclic AMP. Thus, according to pharmacologic criteria and the results of cell separation, the abnormal suppressor activity in this patient appeared identical to that in patients with agammaglobulinemia or common variable immune-deficiency disease.[4,7,8]

Effect of Phenytoin Withdrawal

On admission the number of circulating B lymphocytes bearing surface immunoglobulin was greatly reduced; 10 weeks after drug withdrawal their proportion had increased to a value of 2.5 per cent. In parallel, serum levels of immunoglobulin rose to within the normal range (Table 1).

Spontaneous suppressor cells were present until two to three weeks after the discontinuation of drug therapy, and then disappeared. Plaque-forming-cell responses began to rise and reached normal levels 10 weeks after drug withdrawal. More important, pokeweed mitogen no longer suppressed the response by plaque-forming cells but enhanced it, as in the normal controls and the controls given phenytoin (Table 2).

Since discharge the patient has been examined at regular intervals for 18 months. He has remained healthy, with normal immunoglobulin levels, no spontaneous suppressor-cell activity, and normal responses by plaque-forming cells. The phenytoin has not been reinstituted.

DISCUSSION

Among the numerous side effects of phenytoin, mild to severe abnormalities of the immune system are observed in up to 70 per cent of patients. These include lymphoma-like lesions and hypersensitivity syndromes with exfoliative dermatitis, lupus-like manifestations, lymphotoxic autoantibodies, and lymphopenia, as well as global or selective defects in cell-mediated immunity.[9-19] The most frequent aberration is the development of IgA deficiency, which occurs most regularly in young children given the drug.[20-23] Although several biochemical mechanisms have been suggested as causes of the side effects,[12,24,25] the aberrations may stem from a primary immune response to self determinants altered by the drug. This possibility is consistent with the variation in the expression of side effects, the lack of correlation between the drug dose and the development of the abnormalities, the suggested linkage of the expression of side effects to histocompatibility genes, and the ability of the drug to bind to autologous proteins and act as a potent hapten.[11,13,20,26-28]

In the patient described in this report, a hypersensitivity reaction with hypogammaglobulinemia developed during phenytoin therapy. Hypogammaglobulinemia and the depletion of circulating B lym-

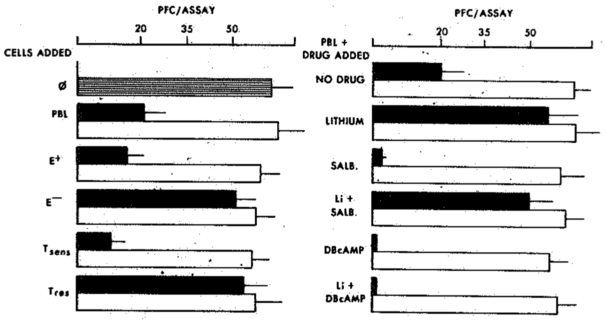

Figure 1. Spontaneous Suppressor-Cell Activity in the Patient (Solid Bars) and a Control (Open Bars).

Fresh peripheral-blood lymphocytes (PBL), purified T lymphocytes (E^+), non-T cells (E^-), and theophylline-sensitive (T_{sens}) and theophylline-resistant (T_{res}) T-cell subpopulations were mixed with cultured normal cells containing 56±4 (mean ±S.D.) ovalbumin-specific plaque-forming cells (PFC) per 2.1×10^4 cells. For the pharmacologic characterization of the spontaneous suppressor-cell activity, the following drugs were added to these cell mixtures: lithium chloride (Li), 3×10^{-4} M; salbutamol (SALB), 10^{-5} M; and dibutyryl cyclic AMP (DBcAMP), 10^{-8} M. The number of residual plaque-forming cells in the cell mixtures was counted after one hour of incubation at 37°C.

Ø denotes controls in the absence of added cells.

phocytes were accompanied by the expression of abnormal, spontaneous suppressor T-cell activity. Three weeks after drug withdrawal, suppressor activity ceased and humoral immunity returned to normal within 10 weeks. Cell separation and pharmacologic studies indicated that the phenytoin-induced suppressor-cell activity was indistinguishable from the activity described in patients with antibody deficiency.[4,7,8,29]

The presence of abnormal T-suppressor-cell function in many patients with antibody deficiency may suggest a pathogenetic role for abnormal T cells in inducing or maintaining arrests in B-cell differentiation.[1-4] However, it is still unclear whether the measurement or elicitation of abnormal suppressor activity in vitro is indeed related to the activity in vivo. In bursectomized birds, abnormal suppressor T cells can produce the disease when transferred into normal birds.[30] Our findings of what appears to be a drug-dependent antibody deficiency and concomitant expression of an abnormal suppressor T-cell activity strengthen the argument for a role of suppressor T cells in the pathogenesis of some disorders of B-cell immunity.

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SULFASALAZINE-INDUCED EXACERBATION OF ULCERATIVE COLITIS

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SULFASALAZINE is used extensively to treat ulcerative colitis. Maintenance therapy with sulfasalazine decreases the frequency of relapse, and the drug has also been used to treat mild to moderate flare-ups of ulcerative colitis.[1,2]

We report studies in a patient with ulcerative colitis in remission who had unequivocal endoscopic and histologic evidence of relapses after oral and rectal challenges with sulfasalazine. Both challenges also provoked the systemic manifestation of iridocyclitis.

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