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Research Article

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ABSTRACT The human inducer ($T4^+$) and reciprocal cytotoxic/suppressor ($T5^+/T8^+$) subsets have been defined by monoclonal antibodies. In the present study, we examined the relationship of naturally occurring anti-T cell autoantibodies found in patients with active juvenile rheumatoid arthritis (JRA) to these subsets. In one approach, normal T cells were treated with anti-T4 or anti-T8 to eliminate the corresponding subset of cells and then analyzed for reactivity with JRA sera. It was found that JRA sera were reactive with only 15% of an enriched cytotoxic/suppressor population, whereas they reacted with 37% of an enriched inducer population. In reciprocal studies, JRA^+ T cells were eliminated with JRA sera and complement and the residual T cells (JRA^-) reacted with monoclonal antibodies and indirect immunofluorescence on a fluorescence-activated cell sorter. As expected, the JRA sera and complement treatment of unfractionated T cells markedly diminished the $T4^+$ subset, whereas there was a concomitant increase in T cells reactive with anti-T5 and anti-T8. A similar diminution in $T4^+$ T cells was found in the circulating peripheral T cell compartment of patients with active JRA who possessed the JRA antibody.

Functional studies demonstrated that removal of the JRA^+ population of T cells diminished phytohemagglutinin and soluble antigen proliferative responses, both of which were previously shown to be functions of $T4^+$ T cells. More importantly, in the absence of JRA^+ T cells, pokeweed mitogen-stimulated immunoglobulin production was markedly enhanced, despite the concomitant increase in $T5^+/T8^+$ cytotoxic/suppressor cells. These results suggest that the JRA serum may define a Qal-like antigen found predominantly on the human inducer population which could activate suppressor and/or other feedback regulatory cells.

INTRODUCTION

The human peripheral T cell population has been shown to consist of distinct subsets of cells by using heteroantisera, autoantisera, and hybridoma antibodies (1–13). In earlier studies, we demonstrated that 20–30% of T cells were reactive with anti-TH_2 heteroantisera (TH_2^+), while 70–80% were unreactive (TH_2^-) (2). The former population contained both cytotoxic effector and mature suppressor cells, whereas the latter contained the helper population (3). Moreover, the TH_2^- subset was itself heterogeneous, since a fraction of the TH_2^- cells, but not TH_2^+ cells, were reactive with an antibody found in the serum of patients with juvenile rheumatoid arthritis (JRA^+)[1] (6). This JRA^+ subset appeared to exert immunoregulatory influence on B cell immunoglobulin secretion (4, 5).

Recently, a series of monoclonal antibodies to human T cell surface antigens have been developed that are capable of defining human T cell subsets. One mono-

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[1] *Abbreviations used in this paper*: Con A, concanavallin A; FACS, fluorescence-activated cell sorter; G/H FITC, fluorescein-conjugated $F(ab')_2$ fragment goat anti-human Ig; JRA, juvenile rheumatoid arthritis; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SLE, systemic lupus erythematosus.

clonal antibody, termed anti-T4, is reactive with the majority of TH_2^- cells and defines the inducer (helper) cell for T-T and T-B interactions in man (10, 11). Two other monoclonal antibodies, termed anti-T5 and anti-T8, define ~20–30% of peripheral T cells, respectively, which contain the entire TH_2^+ cytotoxic/suppressor population (12, 13). These two populations, $T4^+$ and $T5^+/T8^+$, were shown to detect nonoverlapping mature T cell subsets.

In the present study, we sought to better characterize the anti-T cell antibodies in patients with active JRA and compared their specificity to monoclonal anti-T cell antibodies. The studies to be described below show that sera from patients with JRA contain T cell specific antibodies reactive with a portion of the $T4^+$ inducer T cell subset and a small component of the $T8^+$ suppressor T cell population. Functional studies demonstrate that removal of JRA^+ cells with antibody and complement results in a three- to sixfold enhancement of immunoglobulin production in a pokeweed mitogen-driven system. Both the cellular expression of antigen(s) defined by JRA autoantibody and augmentation of immunoglobulin synthesis suggests that JRA antisera might define the equivalent of a Qal-like antigen found on the inducer population for the suppression and/or feedback regulatory subset in man (14–16).

METHODS

Patient materials

Peripheral blood was obtained from patients with JRA studied at Children's Hospital Medical Center, Boston, Mass., and compared to that of normal, healthy volunteers. Only those children for whom a positive diagnosis of JRA was confirmed were included in the study. The criteria for classification as active or inactive disease were based on objective findings by physical examination. Thus, children with at least one swollen joint were considered to have active disease, whether or not they had other physical abnormalities, such as limitation of motion, signs of synovitis, or joint deformities. Patients in remission had normal results on physical examination with full range of motion in all joints and were without evidence of joint involvement. Patients with systemic lupus erythematous (SLE) were studied at the Arthritis Branch of the National Institute of Arthritis, Metabolism, and Digestive Disease, and clinical activity was assessed at the time of blood drawing by two physicians on the basis of signs and symptoms (active rash, serositis, arthritis, active central nervous system disease, and active renal disease). The active patients in this study had at least three of the above criteria of activity. In addition, they all had high titers of antibodies to native DNA. No patients were receiving steroid therapy or other immunosuppressive agents during the time of study.

Sera

Sera from 50 patients with JRA were available for analysis. After filtration through a 0.45 grid membrane, sera were frozen and stored in a small aliquot at −80°C. Before use, the sera were heated to 56°C for 30 min and centrifuged at 100,000 *g* for 20 min to remove aggregated immunoglobulin (Ig)G. These sera were examined for reactivity on T and B cells using a fluorescence-activated cell sorter (FACS-I) (Becton, Dickinson & Co., Rutheford, N. J.) and indirect immunofluorescence as previously described (3).

Isolation of lymphocyte populations

Human peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Unfractionated mononuclear cells were separated into surface Ig^+ (B) and Ig^- (T plus Null) populations by Sephadex G-200 anti-$F(ab')_2$ column chromatography as described (17). T cells were recovered by E rosetting the Ig^- population with 5% sheep erythrocytes (Microbiological Associates, Walkersville, Md.). The rosetted mixture was then layered over Ficoll and the E^+ (T cells) pellet was treated with 0.155 M NH_4Cl for 5 min at room temperature. This recovered T cell population was >95% E rosette positive and <2% EAC rosette positive by using standard methods (18).

The surface Ig^+ (B) population was obtained from the Sephadex G-200 column after elution with a solution of gamma globulin as described (17). Normal human macrophages were obtained from the mononuclear population by adherence to plastic at 37°C overnight. The adherent population was detached by washing cold serum-free medium containing 2.5 mM EDTA. Greater than 85% of these cells ingested latex particles.

Monoclonal antibodies

Four monoclonal antibodies, termed anti-T3, anti-T4, anti-T5, and anti-T8, were used in this study. Their production and characterization was described elsewhere (9–13). In brief, anti-T4 has been shown to react with 55–60% of peripheral T cells, representing the human inducer (helper) population, whereas anti-T5 and anti-T8 defined ~20–30% of T cells, respectively, with cytotoxic/suppressor functions. Since anti-T4 and anti-T8 were of the IgG_2 subclass and fixed complement, they were employed for complement-mediated lysis. A monoclonal antibody, termed anti-T3 and reactive with 100% of peripheral T cells, was used to enumerate T cells (9). It should be noted that all four monoclonal antibodies were restricted in their cellular expression to normal lymphocytes of T lineage.

Analysis of normal T lymphocytes with JRA sera and monoclonal antibodies

10^6 T or B lymphocytes were treated with 0.15 ml JRA sera at a 1:5 dilution, or alternatively, with 0.15 ml of monoclonal antibodies anti-T3, T4, T5, or T8 at a 1:250 dilution. Subsequently, cells were incubated at 4°C for 30 min with 0.15 ml of a 1:20 dilution of fluorescein-conjugated $F(ab')_2$ fragment goat anti-human Ig (G/H FITC) (N. L. Cappel Laboratories Inc., Cochranville, Pa.) in the case of the JRA sera, or with 0.15 ml of a 1:40 dilution of fluorescein-conjugated goat anti-mouse IgG (Meloy Laboratories, Inc., Springfield, Va.) in the case of monoclonal antibodies at 4°C for 30 min.

Next, the cells were centrifuged, washed three times, and then analyzed on the FACS-I as described (3). Background staining was obtained by substituting a 0.15-ml aliquot of normal AB serum for JRA sera or a 1:250 dilution of control ascites from a CAF_1 mouse injected intraperitoneally with a nonproducing hybridoma for specific monoclonal antibodies.

Complement-dependent lysis of lymphocytes with JRA sera

2×10^7 E^+ lymphocytes were incubated in 1 ml of JRA sera (1:5 dilution) containing anti-T cell antibody for 50 min at 4°C. Then, an equal volume of rabbit complement was added to each tube, and the cells were incubated for an additional 3 h at 15°C. The lysed cells were then washed and subsequently placed in final media and cultured overnight at 37°C in 5% CO_2 in a humid atmosphere before cytofluorographic analysis or functional studies.

Complement-dependent lysis of lymphocytes with monoclonal antibodies

E^+ lymphocytes (2×10^7) were treated with either anti-T4 or anti-T8 monoclonal antibodies and rabbit complement. Briefly, 2×10^7 cells were incubated with 1 ml of antibodies at a 1:250 dilution for 1 h at room temperature. Then, 0.3 ml rabbit complement was added and incubated for another hour in a 37°C shaking water bath. After lysis, cells were cultured overnight as described.

Functional studies

Proliferative studies. The mitogenic response of 10^5 unseparated T and T cells preselected with JRA sera and complement (JRA^- T cells) was tested in microculture to an optimal dose of concanavallin A (Con A) (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) and phytohemagglutinin (PHA) (Burroughs-Wellcome Co., Research Triangle Park, N. H.) as described (10). Alloantigen proliferative response was measured concurrently for these same populations by stimulating with mitomycin C-treated Laz 156, an Epstein-Barr virus transformed human B lymphoid cell line. Proliferation to tetanus toxoid (Massachusetts Department of Public Health Biological Laboratories, Jamaica Plain, Mass.) and mumps antigen (Microbiological Associates) were tested as described (10) using 10 μg/ml final concentration and at a 1:20 dilution, respectively. Macrophages were added to all populations at a 5% final concentration at the initiation of in vitro cultures. Mitogen-stimulated cultures were pulsed after 4 d with 0.2 μCi of [^{3}H]TdR (tritiated thymidine) (1.9 Ci/mM sp act) (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) and harvested 18 h later on a MASH II apparatus (Microbiological Associates). [^{3}H]TdR incorporation was measured on a Packard scintillation counter (Packard Instrument Co., Inc., Downer's Grove, Ill.). Background [^{3}H]TdR incorporation was obtained by substituting media for mitogen. Soluble and cell surface alloantigen cultures were pulsed after 5 d with [^{3}H]TdR for 18 h, harvested, and counted as above.

Con A activation of suppressor cells. Whole mononuclear cells were activated with 20 μg Con A/10^6 cells at a concentration of 10×10^6 cells/ml for 10 min. The cells were then diluted to 2×10^6 cells/ml in RPMI 1640 containing 20% fetal calf serum (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), 1% penicillin-streptomycin, 200 mM L-glutamine, 25 mM Hepes buffer (Microbiological Associates), and 0.5% sodium bicarbonate. These Con A-activated cells were cultured upright in 25 cm^2 surface area tissue culture flasks (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for 48 h at 37°C in a humid atmosphere containing 5% CO_2. Untreated cells were cultured in an identical fashion. 5×10^4 of either Con A-activated or untreated T lymphocytes were then added to 10^5 of autologous lymphocytes at the initiation of pokeweed mitogen (PWM) driven IgG secretion.

Measurement of secreted IgG in vitro. Unfractionated and separated populations of lymphocytes were cultured in round bottom microtiter culture plates (Linbro Chemical Co., Hamden, Conn.) at 37°C in a human atmosphere with 5% CO_2 for 7 d in RPMI 1640 supplemented with 20% fetal calf serum, 20 mM L-glutamine, 25 mM Hepes buffer, 0.5% sodium bicarbonate, and 1% penicillin-streptomycin. To determine the effect of T cell subpopulation on secretion of IgG by plasma cells, 5×10^4 unfractionated T cells, or unfractionated T cells treated with JRA sera and complement, were added to 5×10^4 B cells in a volume of 0.1 ml. To this was added 0.1 ml of PWM (Gibco Laboratories, Grand Island Biological Co.) at a 1:50 dilution. Control cultures contained PWM and 10^5 B cells alone, 10^5 unfractionated T cells alone, or 10^5 T cells treated with JRA sera and complement alone. To assess the possibility of unseparated T cells containing suppressor-inducing populations, 2.5×10^4 unseparated T cells were added to a mixture of 2.5×10^4 unseparated T cells treated with JRA sera and complement, and 5×10^4 B cells or 2.5×10^4 $T4^-$ T cells were added to a mixture of 2.5×10^4 treated with JRA sera and complement, and 5×10^4 B cells or 2.5×10^4 $T4^-$ T cells were added to a mixture of 2.5×10^4 unseparated T cells and 5×10^4 B cells, or a mixture of 2.5×10^4 unseparated T cells treated with JRA sera and complement and 5×10^4 B cells. On day 7, cultures were terminated and supernates were harvested.

IgG secretion into supernates was determined by solid-phase radioimmunoassay using a monoclonal antibody directed at the Fc portion of human γ heavy chain (anti-γFc) (gift of Dr. Victor A. Raso, Sidney Farber Cancer Institute). In brief, 0.1 ml of a 1:20,000 dilution of anti-γFc was placed in flat bottom flexible microtiter plates (Cooke Engineering Co., Alexandria, Va.) and incubated at 4°C for 2 h. Subsequently, the unbound antibody was removed from the plates, which were then further incubated with 1% bovine serum albumin in phosphate-buffered saline for an additional hour at 20°C. Wells were next washed twice in phosphate-buffered saline and then 0.025 ml supernates and 0.025 ml ^{125}I-labeled purified IgG were added to each well. The plates were incubated for 17 h at 4°C and then washed several times with phosphate-buffered saline. Individual cells were counted in a gamma counter and results compared with a standard curve prepared with purified human IgG. This assay allowed for the detection of 100–60,000 ng IgG/ml of each culture supernate.

Statistical analysis

Statistical significance was examined by the Student's *t* test.

RESULTS

Reactivity of JRA sera with normal E^+ and Ig^+ lymphocytes

The sera from patients with JRA were analyzed for T cell-specific antibodies by indirect immunofluorescence on the FACS. Only sera of six patients who were experiencing severe disease activity at the time of blood sampling contained antibodies reactive with ~20–50% (average, 27%) of E^+ lymphocytes (Table I). Those individuals who were defined as severe active exhibited at least two of the following systemic symp-

TABLE I
Reactivity of JRA Sera with T Cell Subsets Defined by Monoclonal Antibodies

Antibodies used for analysis	Unseparated T cells	$T4^-$ T cells (suppressor T enriched)	$T8^-$ T cells (inducer T enriched)
	%	%	%
Monoclonal antibodies			
T3	93	92	94
T4	61	<5	92
T8	29	91	<3
Autoantibodies			
JRA I	21	11	30
JRA II			
1:5	49	32	64
1:50	20	15	31
JRA III	35	10	40
JRA IV	22	14	30
JRA V	19	12	34
JRA VI	24	14	31
Mean JRA reactivity	27	15	37

JRA sera were diluted at a 1:5 dilution. JRA II serum was also used at a 1:50 dilution. Values were expressed as average of three different experiments.

toms: polyarticular synovitis, high fever, skin rash, and irilocyclitis. In no case were sera reactive with Ig^+ B cells. All six sera had a plateau of staining at serum dilutions from 1:5 to 1:10 and JRA II serum also had another plateau of staining from 1:40 to 1:80. Other sera from patients with mild or no disease activity were reactive with <10% of E lymphocytes. A representative FACS pattern is shown in Fig. 1. The serum from patient JRA-1 was maximally reactive with 21% of normal E^+ lymphocytes at a 1:5 dilution. As shown in Fig. 1B, the serum was unreactive with Ig^+ lymphocytes at identical dilutions.

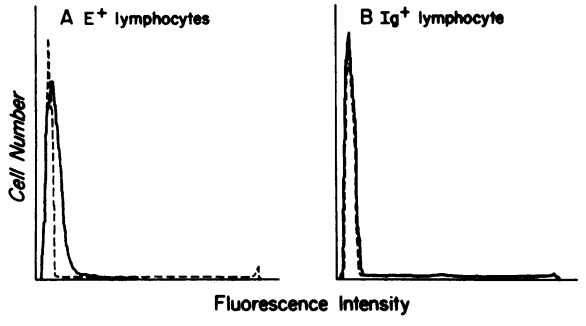

FIGURE 1 Immunofluorescence profile of normal E^+ and Ig^+ lymphocytes after incubation with serum from patient JRA-1 and G/H FITC. (A) The JRA serum was reactive with 21% of normal E^+ lymphocytes at a 1:5 dilution. In contrast, it was unreactive with Ig^+ lymphocytes (B) at identical dilutions. Background fluorescence staining was obtained by incubation each population with an identical dilution of normal AB serum and G/H FITC (dotted line).

Reactivity of JRA sera with T cell subsets defined by monoclonal antibodies

To determine whether JRA sera were reactive with specific subsets of human T lymphocytes, a suppressor enriched population ($T4^-$ T cells) was obtained by lysis of the $T4^+$ population, and an inducer enriched population ($T8^-$ T cells) was prepared by lysis of the $T8^+$ population. Unfractionated T cells, suppressor-enriched, and inducer-enriched T cells were then reacted with an optimal dilution of JRA sera, washed, developed with G/H FITC, and characterized on the FACS by indirect immunofluorescence as previously described. As shown in Table 1, JRA sera reacted with ~37% of the $T8^-$ inducer T cell subset, but only 15% of $T4^-$ T suppressor cell subsets.

These results demonstrated that active JRA sera containing anti-T cell antibodies are predominantly reactive with a portion of the T4 inducer T cell subset and a small fraction of the $T8^+$ cytotoxic/suppressor T cell subset.

Reactivity of monoclonal antibodies with T lymphocytes preselected with JRA sera and complement (JRA^- T Cells)

In reciprocal studies, normal T cells were treated with JRA antibodies and complement, and residual T cells (JRA^- T cells) were tested for reactivity with anti-T3, T4, T5, and T8 on the FACS by indirect immunofluorescence. Preliminary studies showed that JRA sera used in these studies lysed ~20–30% of T cells at identical dilutions used in the FACS study. In control experiments, T cells were treated with the same dilution of normal AB serum and complement. Table II

TABLE II
Reactivity of Monoclonal Antibodies with Unseparated T Cells and T Cells Preselected with JRA Sera and Complement

Monoclonal antibody	Unseparated T cells	JRA^- T cells
anti-T3	94±1	93±1
T4	58±2	44±2*
T5	20±1	30±1*
T8	27±1	38±1*

Unseparated T cells were treated with AB serum and complement. JRA^- T cells are treated with JRA sera and complement. Results are expressed as mean±SEM of six individual experiments using sera derived from different patients and different AB type persons.
* Significant differences $P < 0.05$ based on 95% of confidence limits between unseparated T cells and JRA^- T cells.

summarizes the reactivity of monoclonal antibodies with unseparated T cells and T cells preselected with JRA sera and complement. 94±1% of the unseparated T cell population was reactive with T3, whereas 58±2%, 20±1%, and 27±1% were reactive with T4, T5, and T8, respectively. In contrast, the results were considerably different for the JRA^- T cell subpopulation. The fraction of T cells reactive with T4 decreased from 58±2% to 44±2% ($P < 0.05$). In contrast, the fraction reactive with T5 and T8 increased from 20±1% to 30±1% ($P < 0.05$) and from 27±1% to 38±1% ($P < 0.05$), respectively. These findings suggest that anti-T cell antibodies seen in patients with active JRA are predominantly reactive with the $T4^+$ T cell subset (inducer population).

FACS analysis of lymphocytes from patients with JRA

To determine whether there were concomitant alterations in T cell subsets of patients with JRA, we studied the surface characteristics of lymphocyte subpopulations from patients with active and inactive JRA and compared them to those of normal, healthy individuals and those of active SLE patients (Table III). The normal lymphocyte population is comprised of 67 ±3% T cells, as determined by reactivity with anti-T3. Moreover, some lymphocytes are reactive with anti-T4 (41±2%), which defines inducer T cells, and smaller percentages are reactive with anti-T5 (20±1%) and anti-T8 (22±1%), which define suppressor T cells. However, as shown in Table III, in active JRA, there is a decrease in the T4 population (34±2%) and an increase in the T5 (28±1%) and T8 (31±1%) populations; in inactive JRA, on the other hand, lymphocyte populations are virtually the same as in normals. As a control, we also had examined the T cell subsets in patients with active SLE, as previously described (19). In active SLE, there was the diminution of the total T cell population and the decreased number of $T5^+/T8^+$ T cell subset, indicating that the T cell subsets of active JRA were clearly different from those of active SLE.

TABLE III
Cell Surface Characteristics of T Cells in JRA Patients and Normal Controls

		Reactivity with monoclonal antibodies			
	Number	anti-T3	anti-T4	anti-T5	anti-T8
Normals	30	67±3	41±2	20±1	22±1
active JRA	5	66±3	34±2*	28±1*	31±1*
inactive JRA	5	63±4	43±1	19±2	21±2
active SLE	14	51±3*	40±3	9±1*	11±1*

Results are expressed as mean±SEM.
* Significant differences $P < 0.05$ on the basis of 95% of confidence limits between patients and normal control groups.

Functional characterization of T cells preselected with JRA sera and complement

Proliferative studies: mitogen, soluble antigen, and cell surface antigen responses. Proliferative response of T cells preselected with JRA sera and complement (JRA^- T cell population) was compared with the response of unseparated T cells (AB sera and complement). These populations were supplemented with 5% macrophages before in vitro culture. The control T cell population and JRA^- T cell population were then stimulated with PHA, Con A, soluble antigens, and alloantigens to assess their in vitro proliferative responses. As shown in Table IV, some differences in response to PHA and soluble antigens were obtained with control T and JRA^- T cell populations. For example, after preselection with JRA sera and complement, the proliferative response of residual T cells to PHA, soluble antigens, tetanus toxoid, and mumps antigen was decreased in comparison with the response of control T cells ($P < 0.05$). On the other hand, the proliferative response of these populations to Con A and to alloantigen in mixed lymphocyte culture was identical to the untreated T cells.

PWM-stimulated IgG synthesis. The most striking effect of JRA sera was seen in a PWM-stimulated IgG system when lymphocytes from normal individuals were separated into T and B lymphocytes and the T lymphocytes were then treated with JRA sera and complement. PWM-stimulated IgG synthesis by B cells co-culturing with T cells preselected with JRA and complement (JRA^- T cell population) was compared with that of B cells co-culturing with unseparated T cells (AB sera and complement). As shown in Fig. 2, as ex-

TABLE IV
Proliferative Response of Unseparated and JRA^- T Cells to Mitogens, Soluble Antigens, and Alloantigens

	Unseparated T cells	JRA^- T cells
PHA	84,858±5,341	57,787±5,897*
Con A	62,166±3,600	74,400±8,062
Tetnas toxoid	11,456±838	7,757±799*
Mumps	19,921±1,142	13,204±893*
Laz 156	73,448±4,504	72,504±3,851
Media	714±77	970±97

Unseparated T cells, T cells treated with AB serum + C′; JRA^- T cells, T cells treated with JRA serum + C′. Values are expressed as the mean±SE of five individual experiments using sera derived from different patients.
* Significant differences ($P < 0.05$) on the basis of 95% of confidence limits between unseparated T cells and JRA^- T cells.

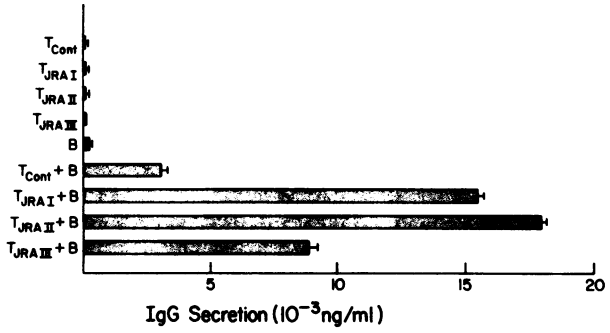

FIGURE 2 Influence of JRA⁻ T cell subset on B cell IgG secretion. T cells with or without JRA sera treatment, B cells, and autologous T-B combinations were cultured in the presence of PWM for 7 d. Subsequently, culture supernates were harvested and IgG secretion was quantitated by radioimmunoassay. Tcont, unseparated T cells treated with AB serum and complement; TJRA I-III, T cells treated with JRA I-III sera and complement. Results are representative of three experiments performed.

pected, there was little PWM-stimulated IgG synthesis by B or T cells alone or by T cells preselected with JRA and complement alone. However, when control T cells were added to autologous B cells, 3,000±28 ng/ml of IgG synthesis was obtained. In contrast, B cells cocultured with JRA⁻ T cell populations exhibited a three- to sixfold increase in IgG synthesis, as opposed to those with unseparated T cells. These results suggest that the changes might be due to the elimination of a regulatory influence by the missing JRA⁺ cells. To further clarify this hypothesis, the following experiments were performed. As shown in Fig. 3, ~5,800±100 ng/ml of IgG was secreted by a mixture of unseparated T cells and B cells activated by PWM, whereas with JRA⁻ T cells and B cells, 17,000±800 ng/ml of IgG was secreted during the 7-d culture after PWM stimulation. In contrast, after adding control T cells to JRA⁻ T cells cocultured with B cells, the high level of IgG production of the JRA⁻ T and B cell combination was reduced to that found with the unseparated T and B cell mixture. These findings suggest that the JRA⁺ subset was contained within the unseparated T cell population and exerted a regulatory effect in the B cell population. To investigate these findings, several additional mixing studies using autologous lymphocyte combinations were performed. Prior studies showed that the T4⁻ subset contained mature suppressor cells (13). Thus, as expected, when T4⁻ T cells were added to unseparated T cells cocultured with B cells, IgG secretion by B cells were suppressed to 1,800±100 ng/ml of IgG secretion. In contrast, when T4⁻ T cells were added to JRA⁻ T cells co-cultured with B cells, IgG secretion by B cells could not be effectively suppressed. These results imply that unseparated T cells contain a suppressor inducer population and that in the absence of the T4⁺, JRA⁺ T cell populations, no induction or activation of suppressor T cells to elicit a strong suppressive effect occurs.

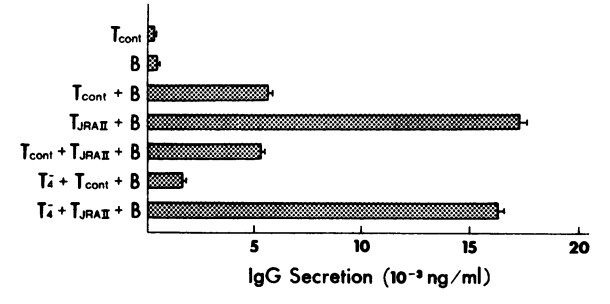

FIGURE 3 Influence of unseparated and T4⁻T cells on B cell IgG secretion of JRA⁻ T and B cell combinations. Unseparated T cells with a mixture of JRA⁻ T and B cells or T4⁻ T cells with a mixture of unseparated T and B cells and with a mixture of JRA⁻ T and B cells were cultured in the presence of PWM for 7 d. Subsequently, culture supernates were harvested and IgG secretion was quantitated by radioimmunoassay. Tcont, unseparated T cells treated with AB serum and complement; TJRA II, T cells treated with JRA II sera and complement; T4⁻, T cells treated with T4 and complement. Results are representative of three experiments performed.

Con A-induced suppressor activity. To determine whether JRA⁺ T cells were essential for the generation of T suppressor activity or suppressor effector activity, T cells were selected with JRA sera and complement either before or after stimulation by Con A and were then added to 10^5 whole lymphocytes to detect PWM-stimulated IgG synthesis. Table V shows that preselection with JRA and complement does not contribute to the generation of either T suppressor activity or suppressor effector activity. These results suggest that JRA⁺ T cells are not required for the generation of Con A-induced T suppressor activity.

DISCUSSION

In the present study, we have characterized the anti-T cell antibody of patients with JRA on normal T lymphocytes and analyzed T cell subsets in these same patients by means of a series of monoclonal antibodies. The sera of patients with severe activity were demonstrated to contain antibodies specific to T cells. Moreover, this reactivity was restricted to ~27% of the normal T cell population. After lysis of normal T cells with JRA sera and complement, the residual T cell population (JRA⁻ T cell population) was studied using a series of T cell subset specific monoclonal antibodies to determine whether a specific subset of human T cells had been eliminated. It was shown that JRA and complement treatment diminished T4⁺ T cell subsets (inducer population) in particular and that there was a corresponding increase in the T cell population reactive with T5/T8 (cytotoxic/suppressor population) subsets. In re-

TABLE V
Effect of JRA Sera Treatment on Con A-induced Suppressor T Cell Function

First culture			Second culture
Treatment of cells before first culture	Treatment of cells after first culture	Con A	Cells added to fresh PWM-stimulated autologous Ly
			ng/ml
AB sera + C′	0	−	10,000±240
		+	400±125
JRA I + C′	0	−	10,200±350
		+	152±42
JRA II + C′	0	−	11,000±400
		+	98±25
JRA III + C′	0	−	10,700±340
		+	340±48
0	AB sera + C′	−	10,412±600
		+	312±48
0	JRA I + C′	−	11,000±420
		+	120±28
0	JRA II + C′	−	10,800±400
		+	184±78
0	JRA III + C′	−	10,000±1,120
		+	242±35

Normal T cells were treated with JRA sera and complement either before or after stimulation by Con A and were then added to autologous whole lymphocytes to detect PWM-stimulated IgG synthesis. Results are representative of three experiments performed. The values are expressed as the mean±SE.

ciprocal studies, normal T cells were first treated with anti-T4 and anti-T8 monoclonal antibodies to eliminate the corresponding subsets of cells and then analyzed for their reactivity with JRA sera. It was shown that JRA sera were reactive with 15% of the $T4^-$ population (enriched cytotoxic/suppressor population) and that, in contrast, 37% of the $T8^-$ population (enriched inducer population) was reactive with JRA sera.

The present study demonstrates that the sera of JRA patients with severe disease activity are mainly reactive with the $T4^+$ inducer population. In addition, it must be noted that some JRA reactivity was found within the $T8^+$ subset. Given that the $T8^+$ subset contains the entire $T5^+$ (TH_2^+) subset and a $T4^-$, $T5^-$ subset as well, it is likely (although not proven) that JRA reactivity in the $T8^+$ subset is restricted to the latter $T4^-$, $T5^-$ subset. Thus, the JRA antigen is expressed mainly on the T4 subset and perhaps a small part on the $T4^-$ and $T5^-$ subset of the T8 population, and may serve to further subclassify these two cell subsets.

The proliferative response of T cells to mitogens, soluble antigens, and alloantigens has also been a valuable aid in the study of lymphocyte function and the dissection of lymphocyte sets. Prior studies suggested that T4 and T5 subsets proliferated equally well to the mitogen stimulation of Con A and to the alloantigen Laz 156 in mixed lymphocyte culture, and the T4 population proliferated maximally to PHA and soluble antigens, whereas the T5 population responded poorly (10, 12). In this respect, it was shown that the populations defined by JRA sera are essential for optimal responses to PHA and soluble antigens, tetanus toxoid, and mumps. These results also support the notion that JRA sera are predominantly reactive with the T4 inducer population.

Earlier studies demonstrated that JRA^+ T cells responded maximally to Con A but not to PHA or allogeneic cells (4). These prior results are discrepant with the present findings and are perhaps related to the use of different techniques. For example, in earlier studies, to separate JRA^+ T cells, T lymphocytes sensitized with JRA sera were rosetted with human erythrocytes previously coated with purified rabbit anti-human light chain antibodies. These techniques have been shown to result in heterogeneous populations and select for some Fc positive lymphocytes including Tγ cells. Recent studies demonstrated that the Tγ enriched population was heterogeneous since anywhere from 5–50% of cells were T3 reactive, 50–90% were M1 reactive (20).

Perhaps more importantly, the functional study of PWM-stimulated IgG production showed that B cells co-cultured with JRA^- T cells exhibited extraordinary enhancement of IgG synthesis as compared with unseparated T cells. This occurred despite the observations that JRA treatment decreased the T4 population (inducer subset) and increased the T5/T8 population (cytotoxic/suppressor subset). On the other hand, the study of Con A-inducer suppressor cell function indicated that JRA^+ T cells are not required for generation of Con A-induced T suppressor activity, previously shown to be mediated by the TH_2^+ ($T5^+$) subset (3, 12). Thus, the participation of JRA^+ T cells in the regulation of IgG production appears to be largely independent of the mature suppressor $T5^+/T8^+$ subset and suggests that the JRA^+ subset may be involved in the induction of a T cell suppressor population. This could provide an explanation for the augmentation of PWM-stimulated IgG synthesis in vitro and ineffective suppression of PWM-stimulated IgG secretion in spite of adding $T4^-$ suppressor cells following elimination of JRA^+ cells.

In this regard, our earlier study showed that patients with active JRA appeared to have autoantibodies, lacked a specific JRA^+ subset of T cells, and more importantly, had increased B cell production of Ig (4, 5).

The study of lymphocyte subsets in patients with JRA clearly established that in the active stage, JRA patients have high levels of $T5^+/T8^+$ cytotoxic/suppressor cell populations but reduced numbers of T4 inducer T cells. However, due to the reduced number of JRA^+ T cells in these patients, lymphocytes might not induce cells to elicit strong suppressive responses, in spite of high levels of $T5^+/T8^+$ subsets. Therefore, this type of T-T interaction plays a physiological role in the regulation of the in vivo immune response in man. More recently, Eardley et al. (14) and Cantor and co-workers (15, 16) showed that in mice, the maintenance of immunological homeostasis was governed by the Qal feedback inhibitory circuit. These studies indicate that Lyl Qal^+ cells induce Lyl, 2, 3, Qal^+ cells to generate potent feedback inhibitory activity. It was also shown that Qal antigen is expressed by both Lyl and Lyl, 2, 3 cells (21). These JRA antigen systems are also expressed on the $T4^+$ subset, the human analogue of the murine Lyl subset, and a fraction of the $T8^+$ subset. Perhaps the latter represents the human analogue of Lyl, 2, 3 and this antigen defines the feedback inhibitory circuit in man and the analogous Qal antigen system in mice. As for the possibility that prostaglandin inhibitors affect our assay system, it is unlikely. Although three of six patients with JRA used in this study had received salicylate when blood was drawn, the other three JRA patients had received no drugs such as prostaglandin inhibitors.

Earlier studies demonstrated that patients with active SLE had a loss of suppressor T lymphocyte function (22–28). Several studies have emphasized the importance of anti-T cell antibodies in altering T cell number and function in patients with active SLE (7, 8, 28–34). In this regard, recent studies demonstrated that anti-T cell antibodies found in the sera of active SLE patients were selectively reactive with the $T5^+/T8^+$ suppressor T cell population (8) and patients with active SLE had decreased numbers of $T5^+/T8^+$ subsets (19). These data clearly demonstrated that the subset defined by anti-T cell antibodies found in JRA were different from those defined by anti-T cell antibodies of SLE and suggest that the immunoregulatory defect in JRA maps to a completely different site in the suppressor circuit than does the one in SLE.

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