Abnormalities in CD4+ T-Lymphocyte Subsets in Inflammatory Rheumatic Diseases

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The monoclonal antibodies anti-2H4 and anti-4B4 identify the suppressor-inducer (CD4+2H4+) and helper-inducer (CD4+4B4+) subpopulations of CD4 (T4+) lymphocytes, respectively. The cell surface phenotype of peripheral blood lymphocytes and synovial fluid lymphocytes in patients with rheumatoid arthritis and other inflammatory joint diseases was analyzed by use of these and other well-characterized anti-T-cell monoclonal antibodies. In the synovial fluid of patients with rheumatoid arthritis, there was a markedly decreased percentage of T4+2H4+ suppressor-inducer cells (3.1 ± 1 percent) and an increased percentage of T4+4B4+ helper-inducer cells (29.1 ± 9 percent) as compared with the proportions found in the peripheral blood of normal individuals (T4+2H4+: 19.0 ± 6 percent, T4+4B4+: 23.0 ± 7 percent). Moreover, patients with other chronic and acute inflammatory joint diseases exhibited highly similar synovial T-cell findings to those of the patients with rheumatoid arthritis (T4+2H4+: 4.2 ± 3 percent, T4+4B4+: 33.1 ± 9 percent). In contrast, there were no significant differences between the normal control subjects and patients with rheumatoid arthritis in the percentage of T4+2H4+ cells in peripheral blood lymphocytes, nor were there significant differences between normal control subjects, patients with rheumatoid arthritis, and patients with other joint diseases (osteoarthritis, gout, B27+ spondyloarthropathy, and psoriatic arthritis) in the number of T4+4B4+ cells or in the T4/T8 ratio of peripheral blood lymphocytes. However, very low numbers of T4+2H4+ (suppressor-inducer) peripheral blood lymphocytes were seen in a subgroup of patients, including five of seven with Reiter's syndrome and several patients with systemic rheumatic disease syndromes. In addition, although the percentage of T4+2H4+ cells in peripheral blood lymphocytes of patients with osteoarthritis (13.7 ± 7 percent) and gout (14.3 ± 7 percent) was decreased compared with that of normal controls (19.0 ± 6 percent) (osteoarthritis versus normal controls p <0.025), this difference appeared to reflect alterations due to age rather than disease. Consistent with the phenotypic changes observed, synovial T cells were also functionally defective, since autologous mixed lymphocyte reaction-activated T4 cells from the synovial fluid of patients with rheumatoid arthritis failed to exhibit suppressor-inducer activity. The results indicate that diminished proportions of CD4+2H4+ (suppressor-inducer) cells and increased proportions of CD4+4B4+ (helper) cells are a common feature of CD4+ cells in synovial fluid in rheumatoid arthritis as well as a variety of other inflammatory disorders, whereas modest changes in CD4+2H4+ peripheral blood lymphocytes are seen in older individuals and more marked decreases are

observed only in a more selected group of patients. These changes may be of potential functional importance in the regulation of the immune response in a variety of clinical settings.

Rheumatoid arthritis is a chronic, systemic inflammatory disorder of unknown etiology characterized by the manner in which it involves the joints. Unidentified initiating stimuli appear to cause an aberration within the immune system in genetically susceptible persons that may be self-perpetuating. There are multiple abnormalities of both humoral and cellular immunity in patients with rheumatoid arthritis, including circulating rheumatoid factors, hypergammaglobulinemia, cutaneous anergy, and increased numbers of circulating activated lymphocytes [1–4]. More marked changes are demonstrable in affected joints and in the inflamed synovium which, with its complex immune-cell infiltrate, appears like and in many respects can function as an ectopic lymphoid organ [3–6]. The T cells found in synovial tissue and fluid are highly activated and differ in many respects from circulating T cells in patients with rheumatoid arthritis and in normal subjects [4,6–18]. In earlier studies of patients with rheumatoid arthritis, abnormalities in CD4 (T4)/CD8 (T8) ratios of peripheral blood lymphocytes were modest and sometimes difficult to demonstrate, while studies of synovial fluid and tissue lymphocytes have frequently shown a lower T4/T8 ratio than in peripheral blood, with only a slight excess of T4 over T8 cells [8–12]. Moreover, lymphocytes from rheumatoid synovial fluid exhibit numerous abnormalities. They are reported to exhibit abnormalities in the autologous mixed lymphocyte reaction (AMLR) [12–14], are hyperactive in their responses to some mycobacterial and endogeneous antigen [15,16], deficient in suppressor function [12,17–18], and, despite the presence of numerous, apparently activated cells with CD8+ cytotoxic/suppressor phenotype, are able to provide or augment help in some experimental systems for B cell Ig production and for the activation of macrophages [12,17,19]. The cellular basis for these functional T-cell abnormalities is unknown, and it is unclear whether they occur primarily in rheumatoid arthritis, representing unique pathophysiologic mechanisms, or if they are of more general importance in other chronic immune-mediated rheumatic disease syndromes.

We have recently developed the novel monoclonal antibodies anti-2H4 and anti-4B4, which can be used to subdivide human CD4 (T4) cells into distinct and largely reciprocal functional subsets [20,21]. The anti-2H4 antibody defines a subset of T4 cells (T4+2H4+), previously described as the T4+JRA+ subset [22], that induces T8 cells to exert suppressor function in both pokeweed mitogen-driven IgG [20] and antigen-specific antibody production systems [23] and which maximally responds in the AMLR [24,25]. In contrast, the anti-4B4 antibody defines

a reciprocal population of T cells (T4+4B4+) that act to provide helper-inducer function [21]. We have characterized both peripheral blood lymphocytes and synovial fluid lymphocytes in patients with rheumatoid arthritis as well as other inflammatory joint diseases. The results showed that diminished proportions of T4+2H4+ cells and increased proportions of T4+4B4+ cells are a common feature of T4 cells in synovial fluid in rheumatoid arthritis as well as a variety of other acute and chronic inflammatory disorders, whereas changes in peripheral blood lymphocytes are seen in a more selected group of patients.

PATIENTS AND METHODS

Patients. Peripheral blood samples were obtained from 21 patients with classical or definite rheumatoid arthritis according to the criteria of the American Rheumatism Association [26]. These included seven men and 14 women, with a mean age of 56 ± 12 years (mean ± SD), 37 patients with other articular diseases (16 men and 21 women with a mean age of 53 ± 13 years), including osteoarthritis, 17; gout, four; HLA-B27-associated spondyloarthropathy (including ankylosing spondylitis and Reiter's syndrome), 11; and psoriatic peripheral arthritis, five; and 33 healthy control subjects (15 men and 18 women, mean age 40 ± 15 years). Peripheral blood lymphocytes were also obtained from one patient with progressive systemic sclerosis and two patients with primary Sjögren's syndrome.

Synovial fluid samples were obtained from 12 patients with rheumatoid arthritis and six with other inflammatory joint diseases (gout, one; pseudogout, one; inflammatory bowel disease with peripheral arthritis, one; psoriatic arthritis, one; and Reiter's syndrome, two). Peripheral blood lymphocytes were simultaneously obtained from six patients with rheumatoid arthritis, two patients with Reiter's syndrome, and one patient with gout.

Isolation of Lymphocytes. Human mononuclear lymphocytes from heparinized blood and synovial fluid were isolated by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, New Jersey) as previously described [12,20].

Monoclonal Antibodies. Four monoclonal antibodies termed anti-CD4 (T4), anti-CD8 (T8), anti-2H4, and anti-4B4 were used in the current study. Their production and characterization are described elsewhere [20,21,27]. Anti-T4, -T8, -2H4, and -4B4 are available through Coulter Immunology, Hialeah, Florida.

Analysis of Lymphocyte Populations with Single and Two-Color Fluorescence Flow Cytometry. Single-color fluorescence flow cytometric analysis was performed on an Epics C cell sorter (Coulter Electronics, Inc.). Cells were stained with the monoclonal antibodies at a dilution of 1:500 followed by incubation with a F(ab')$_2$ fragment of goat anti-mouse antibody conjugated to fluorescein isothiocyanate (FITC) (Tago Inc., Burlingame, California). Background fluorescence reactivity was determined with control ascites fluid obtained from mice immunized with a non-secreting hybridoma. Two-color analysis was carried out on an Epics C cell sorter using a 488-nm argon laser, gating for lympho-

TABLE I   Cell Surface Characteristics of Lymphocytes in Blood and Synovial Fluid in Patients with Rheumatoid Arthritis or Other Articular Diseases, and Normal Control Subjects*

Disease Group	Sex (F/M)	Age (years)	Percent				
			2H4	4B4	T4+2H4+	T4+4B4+	T4/T8
Blood							
Normal controls (n = 33)	18/15	40 ± 15	54 ± 9	52 ± 13	19.0 ± 6	23.0 ± 7	2.1 ± 1.0
Rheumatoid arthritis (n = 21)	14/7	56 ± 12	49 ± 16	56 ± 18	15.5 ± 8	24.2 ± 9	2.4 ± 1.0
Osteoarthritis (n = 17)	13/4	62 ± 11	47 ± 17	60 ± 16	13.7 ± 7‡	26.7 ± 7	2.2 ± 0.8
Gout (n = 4)	0/4	57 ± 11	43 ± 10	63 ± 13	14.3 ± 7	28.5 ± 8	2.3 ± 0.7
B27+ spondyloarthropathy (n = 11)	3/8	37 ± 13	46 ± 18	49 ± 13	14.4 ± 9	22.3 ± 9	2.3 ± 0.8
Psoriatic arthritis (n = 5)	3/2	52 ± 6	61 ± 14	48 ± 8	15.1 ± 6	21.0 ± 7	2.0 ± 1.2
Synovial fluid							
Rheumatoid arthritis (n = 12)	10/2	49 ± 13	22 ± 11†	66 ± 18‡	3.1 ± 1†	29.1 ± 9§	2.0 ± 1.6
Other (n = 6) Inflammatory joint diseases	2/4	33 ± 11	25 ± 22**	77 ± 9†	4.2 ± 3†	33.1 ± 9‡	ND

ND = not determined.
* Results are expressed as mean ± SD.
† $p < 0.005$ as compared with normal control subjects.
‡ $p < 0.025$ as compared with normal control subjects.
§ $p < 0.05$ as compared with normal control subjects.
** $p < 0.01$ as compared with normal control subjects.

cyte populations, and utilizing FITC-anti-T4, 2H4-phycoery-thrin (PE), and 4B4-PE. For each sample, 10,000 cells were analyzed on a log fluorescence scale as described previously [28]. All analyses were performed without knowledge of patients' clinical status, and after surface labeling with monoclonal antibodies, the cells were immediately applied to the flow cytometer without formaldehyde fixation.

Suppressor Function of Autologous Mixed Lymphocyte Reaction (AMLR)-Activated T Cells. Suppressor function of T4 cells from synovial fluid and peripheral blood in patients with rheumatoid arthritis upon activation during the AMLR was assessed by the addition of AMLR-activated T4 cells to secondary cultures freshly prepared from a healthy single donor. AMLR cultures were prepared in RPMI 1640 supplemented with 10 percent AB serum, 4 mM L-glutamine, 25 mM Hepes buffer (Microbiological Associates), 0.5 percent sodium bicarbonate, and 1 percent penicillin-streptomycin. For primary AMLR cultures, 2×10^6 responder T4 cells were co-cultured with 2×10^6 irradiated (5,000 rads) peripheral blood E⁻ cells in 4 ml total volume of media in 25-cm² culture flasks (Falcon, Oxnard, California). After seven days, cultured cells were layered over Ficoll-Hypaque, centrifuged at 2,000 revolutions/minute for 20 minutes, and then washed three times. For assessing suppressor-inducer function, 2×10^4 AMLR-activated T4 cells were added to 1×10^5 freshly isolated peripheral blood lymphocytes from a healthy single donor in the presence of pokeweed mitogen (1:100) and cultured in round-bottomed 96-well microculture plates in 200 μl/well of media with 20 percent fetal calf serum for seven days as previously described [25]. IgG in culture supernatants was determined by radioimmunoassay.

Statistical Methods. Results are expressed as mean ± SD. Statistical analysis was performed using a two-tailed t test as indicated to calculate p values.

RESULTS

Cell surface antigens of lymphocyte populations from peripheral blood and synovial fluid in patients with rheumatoid arthritis, patients with other joint diseases (osteoarthritis, gout, B27+ spondyloarthropathy, and psoriatic arthritis), and healthy normal control subjects are summarized in **Table I.** There were no significant differences between normal controls, patients with rheumatoid arthritis, and patients with other joint diseases (osteoarthritis, gout, B27+ spondyloarthropathy, and psoriatic arthritis) in the percentage of 2H4-bearing and 4B4-bearing lymphocytes, T4+4B4+ cells, or the T4/T8 ratio of peripheral blood lymphocytes. Although there were no significant differences between normal control subjects and patients with rheumatoid arthritis in the percentage of T4+2H4+ cells in peripheral blood lymphocytes, the percentage of T4+2H4+ suppressor-inducer cells in peripheral blood lymphocytes of patients with osteoarthritis (13.7 ± 7) and gout (14.3 ± 7) was decreased compared with the percentage in our normal control group (19.0 ± 6) (osteoarthritis versus normals, $p < 0.025$). When the normal control subjects were arbitrarily divided into younger (less than 50 years old) and older (50 years old or older) groups, it was found that the older group had a somewhat lower mean value for T4+2H4+ peripheral blood T cells, (14.5 ± 6 percent) compared with (20.5 ± 6 percent), respectively. The values for the patients with osteoarthritis and gout (with mean ages of 62 and 57 years, respectively) did not differ from those of the older control subjects. Taken together, these data suggest an age-associated rather than disease-associated decrease in this T-cell population.
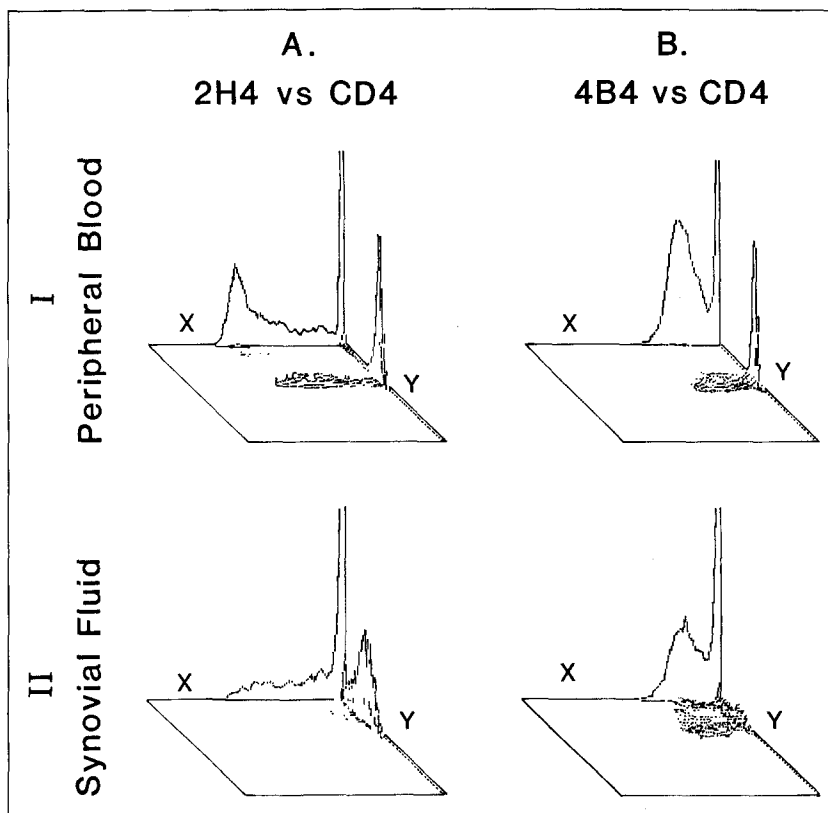
Figure 1. *Profile of peripheral blood lymphocytes (I) and synovial fluid lymphocytes (II) co-expressing either CD4 (T4) and 2H4 or CD4 (T4) and 4B4 antigens in a patient with rheumatoid arthritis. These analyses were done using anti-T4 conjugated to FITC and anti-2H4 and anti-4B4 conjugated to PE.*

In contrast to peripheral blood lymphocytes, synovial fluid lymphocytes exhibited substantial differences in expression of these cell surface antigens. For example, patients with rheumatoid arthritis as well as other inflammatory joint diseases had a marked decrease in total 2H4-bearing cells (22 ± 11 percent and 25 ± 22 percent, respectively) and T4+2H4+ suppressor-inducer cells (3.1 ± 1 percent and 4.2 ± 3 percent, respectively) as compared with the proportions found in peripheral blood lymphocytes. Furthermore, there was a significantly increased percentage of 4B4-bearing cells (66 ± 18 percent and 77 ± 9 percent, respectively) and T4+4B4+ helper cells (29.1 ± 9 percent and 33.1 ± 9 percent, respectively) in synovial fluid as compared with peripheral blood lymphocytes.

Figure 1 shows representative two-color profiles of peripheral blood lymphocytes and synovial fluid lymphocytes co-expressing either T4 and 2H4 or T4 and 4B4 antigens in a patient with rheumatoid arthritis. These analyses were done using anti-T4 conjugated to FITC and anti-2H4 and anti-4B4 conjugated to PE. Log green fluorescence (the T4 subset) is shown along the Y axis and log red fluorescence (the 2H4 subset, A, or the 4B4 subset, B) is shown along the X axis. Cell number is represented by the height of the curve. T4+2H4+ or T4+4B4+ double-positive cells are shown as cell clus-

ters in the center of each panel. In this representative example, 21.1 percent of cells are T4+2H4+ (suppressor-inducer) and 23.4 percent of cells are T4+4B4+ (helper-inducer) among peripheral blood lymphocytes, but only 3.1 percent of cells are T4+2H4+ and 29.4 percent of cells are T4+4B4+ in the synovial fluid of this patient. **Figure 2** shows the percentages of T4+2H4+ cells in peripheral blood and synovial fluid in all groups studied.

Since a value of less than 7 percent represents 2 SD below the mean percentage of T4+2H4+ cells in the peripheral blood of normal control subjects, values below 7 percent were considered to be significantly lower than normal. As shown in Figure 2, only one of 21 patients with rheumatoid arthritis had significantly decreased numbers of T4+2H4+ cells in peripheral blood lymphocytes. This patient, although lacking any clinically significant synovitis, had Felty's syndrome with associated splenomegaly and marked granulocytopenia. The number of T4+2H4+ cells in the peripheral blood lymphocytes of all patients with rheumatoid arthritis without associated disease was within the normal range. These values were not found to correlate with either disease activity or medication (data not shown). Moreover, all patients studied with osteoarthritis, gout, and psoriatic arthritis had normal percentages of T4+2H4+ peripheral blood lymphocytes. Unexpect-
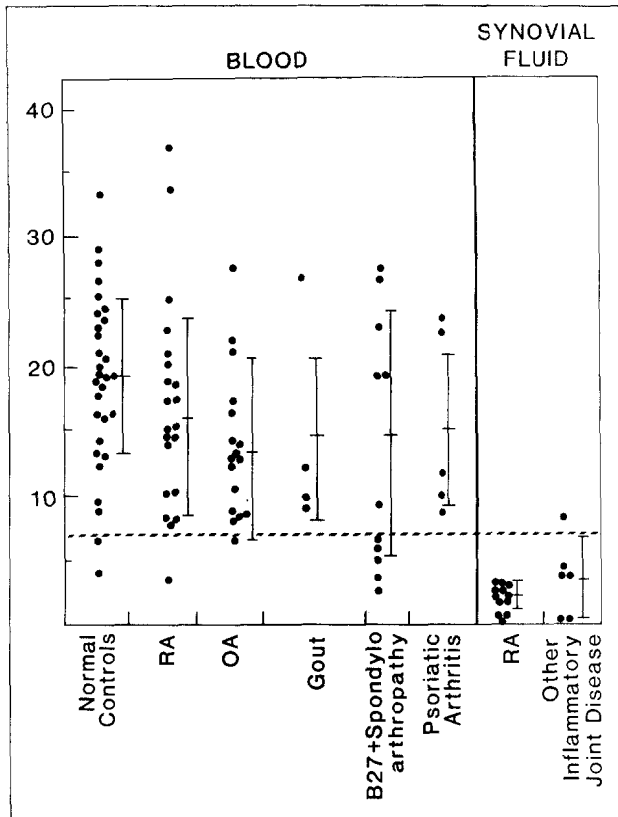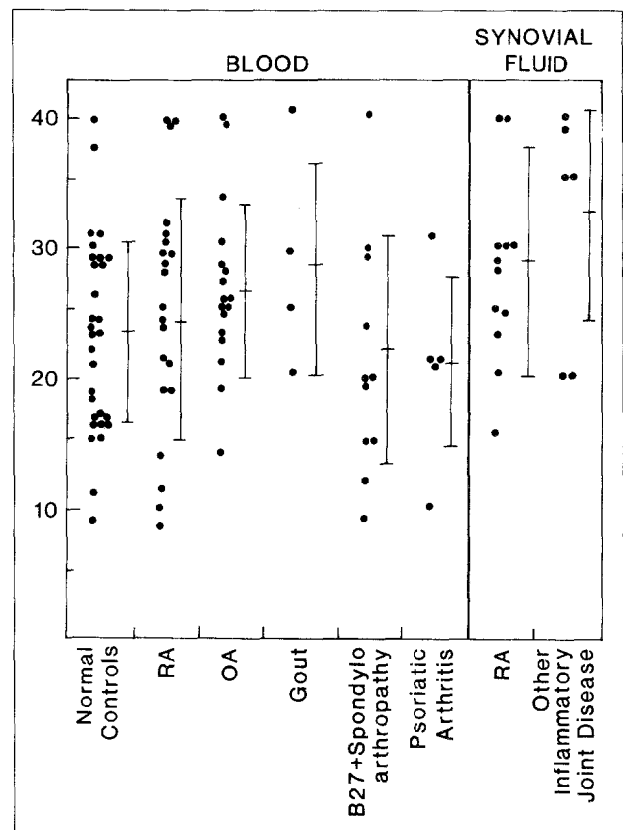
Figure 2. *Percentage of CD4+2H4+ cells in the peripheral blood and synovial fluid of patients and control subjects. The area below the dotted line indicates the lower limit ± 2 SD from the mean percentage of T4+2H4+ cells in the peripheral blood of normal control subjects (7 percent).*

edly, five of 11 patients with B27+ spondyloarthropathy had a significantly decreased number of T4+2H4+ cells in the peripheral blood (Figure 2). A detailed review of these patients' clinical data revealed that of the seven patients in this group with Reiter's syndrome, five had abnormally low numbers of T4+2H4+ cells (mean for the seven patients: 9 ± 8 percent), whereas three of three patients with ankylosing spondylitis had normal values (mean 24 ± 4 percent). Most of these patients were receiving nonsteroidal anti-inflammatory drugs. No association could be made in the patients with Reiter's syndrome between disease activity or medication and the proportion of T4+2H4+ cells, although of the three patients with ankylosing spondylitis, two were taking sulfasalazine and the other had no evidence of clinical activity. Moreover, one patient with progressive systemic sclerosis (5.0 percent T4+2H4+) and two patients with primary Sjögren's syndrome (4.5 percent and 3.0 percent T4+2H4+, respectively) had a significantly decreased percentage of T4+2H4+ cells. By contrast, in the synovi-

al fluid, all 12 patients with rheumatoid arthritis and five of six patients with other inflammatory joint disease, including crystal-induced acute inflammation, had a significantly decreased number of T4+2H4+ cells. The percentages of T4+4B4+ cells in peripheral blood and the synovial fluid in all groups studied are shown in **Figure 3**. All groups had a normal range of T4+4B4+ cells in peripheral blood lymphocytes. In synovial fluid, the patients with rheumatoid arthritis as well as the patients with other inflammatory joint diseases had a significantly higher percentage of T4+4B4+ cells compared with the percentage in peripheral blood (see Table I). Thus, a significant decrease of T4+2H4+ suppressor-inducer cells together with a significant increase of T4+4B4+ helper-inducer cells was found in the synovial fluid of patients with rheumatoid arthritis and other inflammatory joint diseases compared with the peripheral blood of normal subjects or of patients with rheumatoid arthritis or other joint diseases.

Because T cells found in synovial tissue and fluid are known to represent a highly activated population, we

Figure 3. *Percentage of CD4+4B4+ cells in the peripheral blood and synovial fluid of patients and control subjects. In synovial fluid, the patients with rheumatoid arthritis as well as the patients with other inflammatory joint diseases had a significantly higher percentage of T4+4B4+ cells compared with the percentage in peripheral blood.*
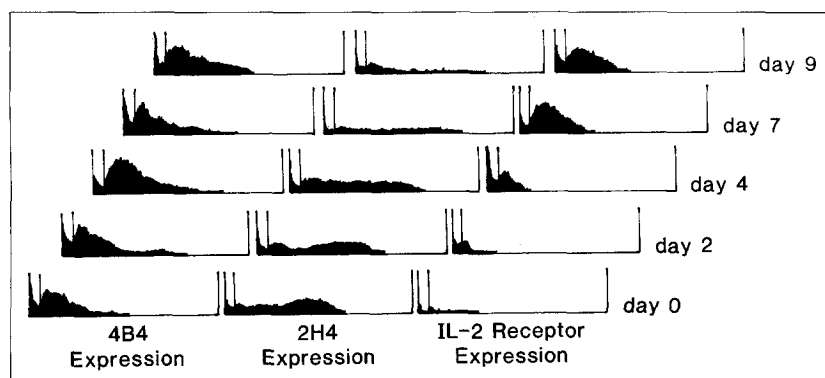
Figure 4. 2H4, 4B4, and IL-2 receptor expression on phytohemagglutinin-activated T4 cells. T4 cells from a normal donor were activated with phytohemagglutinin (0.25 μg/ml) in the presence of 5 percent macrophages for the indicated number of days. Subsequently, the cells were washed four times and were stained with monoclonal antibodies and fluorescein-conjugated F(ab')₂, and cytofluorographic analysis was performed by indirect immunofluorescence on an Epics C cell sorter. Cells (10,000) were analyzed on a log fluorescence scale.

sought to examine whether in vitro activation of T cells might produce alterations in the surface expression of 2H4 similar to those we had observed in vivo. The expression of 2H4 and 4B4 was examined over a nine-day period following activation by the mitogen phytohemagglutinin. T4 cells from a normal donor were activated with phytohemagglutinin (0.25 μg/ml) for four days, after which the culture medium was replaced every two days with medium containing 10 percent T-cell growth factor and 10 percent human serum. As shown in **Figure 4,** at the initiation of culture (Day 0), 2H4 was detected on 51 percent of these cells. On Day 4, the percentage of cells expressing 2H4 was starting to decrease. By this time, the percentage of cells expressing 4B4 and the density of 4B4 expression were both increased. Notably, a diminished number of T4+2H4+ cells were present on Day 7 (23 percent) and Day 9 (21 percent), along with decreased levels of 2H4 antigen on the 2H4+ cells present. IL2 receptor expression was examined in parallel. IL2 receptor expression was minimal on Day 2 but gradually increased. By Days 7 and 9, IL2 receptors were expressed on approximately 60 percent of the phytohemagglutinin-activated T4 cells. As in the representative experiment shown, in most experiments activation of T cells with phytohemagglutinin or concanavalin A ultimately resulted in a gradual decrease in 2H4 antigen expression and increased 4B4 antigen expression. In some donors, however, the 2H4 antigen was stably expressed on concanavalin A- or phytohemagglutinin-activated T cells for up to 21 days of culture (data not shown).

These studies suggest that in vivo T-cell activation may be responsible for diminished 2H4 expression and the decreased numbers of T4+2H4+ cells observed. Further studies, however, will be required to determine the relative contributions of diminished 2H4 antigen expression on T4+ cells that are initially 2H4+ and the possibility that these data might instead reflect a selective growth advantage of T4+4B4+ cells over T4+2H4+ cells under long-term culture conditions or with chronic in vivo stimulation. These two possibilities are not mutually exclusive.

To determine whether the decreased percentage of T4+2H4+ suppressor-inducer cells in synovial fluid was associated with a corresponding functional defect, we utilized a well-defined functional assay system for detection of suppressor-inducer activity [25]. The co-culture of CD4+ T cells activated during an AMLR with fresh pokeweed mitogen-stimulated peripheral blood lymphocytes will induce the CD8+ T cells present in the second culture to suppress B cell Ig secretion in that culture. We therefore examined the suppressor-inducer activity of AMLR-activated T4 cells from both peripheral blood and synovial fluid in patients with rheumatoid arthritis and of AMLR-activated T4 cells from peripheral blood of normal controls. For this purpose, peripheral blood T4 cells from a normal donor and T4 cells from both peripheral blood and synovial fluid in patients with rheumatoid arthritis were stimulated for seven days with autologous peripheral blood non-T cells, then these AMLR-activated T4 cells (2 × 10⁴) were added to 1 × 10⁵ freshly isolated peripheral blood lymphocytes from a healthy single donor to assess their effect on pokeweed mitogen-driven IgG synthesis. As shown in **Table II,** experiments 1 and 2, both AMLR-activated T4 cells from a normal donor and AMLR-activated T4 cells from peripheral blood of patients with rheumatoid arthritis exhibited a strong suppressor activity. In sharp contrast, AMLR-activated T4 cells from the synovial fluid of the same patients had minimal or no suppressor activity. Thus, not only was the suppressor-inducer subset quantitatively diminished in rheumatoid arthritic synovial fluid by phenotypic criteria, but suppressor-inducer activity of T4 cells was correspondingly diminished by functional assay as well. Due to difficulties in obtaining concurrent synovial fluid and peripheral blood lymphocyte samples, only two independent analyses of both phenotypic and functional parameters could be performed.

COMMENTS

The current study utilizes the anti-2H4 and anti-4B4 monoclonal antibodies to characterize the cell surface phenotype of lymphocytes in peripheral blood and synovial fluid

of patients with rheumatoid arthritis and other joint diseases. We found that in the synovial fluid, patients with rheumatoid arthritis had a selectively decreased percentage of T4+2H4+ suppressor-inducer cells and significantly increased percentage of T4+4B4+ helper-inducer cells as compared with the proportions found in peripheral blood. Moreover, patients with other chronic and acute inflammatory joint diseases exhibited highly similar findings. In contrast, in the peripheral blood, most patients with rheumatoid arthritis and other joint diseases had a normal percentage of T4+2H4+ and T4+4B4+ cells compared with the percentage in normal control subjects. Interestingly, one patient with rheumatoid arthritis and Felty's syndrome, two patients with primary Sjögren's syndrome, one patient with progressive systemic sclerosis, and five of seven patients with Reiter's syndrome had significantly decreased percentages of T4+2H4+ cells in the peripheral blood. The basis for the abnormalities in these patients is currently unclear, but might relate to the systemic nature of these syndromes. These observations will require further examination, including evaluation of a much larger number of patients, as well as serial studies. In addition, our current and previous findings also suggest that there are age-related changes in the proportions of these cell populations, unrelated to inflammatory disease.

We had previously reported that patients with chronic progressive multiple sclerosis and patients with active systemic lupus erythematosus (particularly those patients with active renal disease) had a selective decrease in the number of T4+2H4+ suppressor-inducer cells in the peripheral blood [28,29]. Of the patients with rheumatoid arthritis, only the patient with Felty's syndrome had a significantly decreased number of T4+2H4+ cells in her blood. In this regard, two groups have reported that a defect of suppressor T-cell function is present in patients with early active rheumatoid arthritis (duration of active disease less than three months) but not in patients with chronic active rheumatoid arthritis (duration of active disease more than six to 12 months) [30,31]. Since all of our patients with rheumatoid arthritis studied had clearly established disease with a duration of at least three months, it is possible that some patients with very early disease might exhibit a suppressor cell defect that we did not detect in our system in this study. Further longitudinal study including early patients with possible rheumatoid arthritis will be required to clarify these points.

It has been shown that the T4+2H4+ subset of cells proliferates maximally in response to autologous non-T cells but poorly to soluble antigen stimulation [20,25], thus explaining in part our prior observations that predominantly suppressor signals, rather than help for Ig secretion, were generated during the AMLR in the absence of foreign antigen [32]. Moreover, T4+2H4+ cells are responsible for the inducer of suppressor function similar to that previously described for the T4+JRA+ subset, by which T8+ cells are induced or activated to exert sup-

TABLE II Suppressor-Inducer Function of AMLR T4 Cells from Peripheral Blood Lymphocytes and Synovial Fluid in Patients with Rheumatoid Arthritis*

	Experiment 1	Experiment 2
—	8,660 ± 310	7,140 ± 220
AMLR T4$_N$	3,850 ± 260 (56)	2,930 ± 170 (59)
AMLR RA T4$_{PB}$	3,970 ± 180 (54)	3,050 ± 150 (57)
AMLR RA T4$_{SF}$	8,010 ± 270 (8)	7,560 ± 110 (−5)

* T4$_N$ = peripheral blood T4 cells from a normal donor; T4$_{PB}$ = peripheral blood T4 cells from a patient with rheumatoid arthritis; T4$_{SF}$ = synovial fluid T4 cells from a patient with rheumatoid arthritis.
AMLR-activated T4 cells (2 × 10^4) were added to 1 × 10^5 freshly isolated peripheral blood lymphocytes from a normal donor with pokeweed mitogen to assess their immunoregulatory role. Results were expressed as mean of triplicate samples ± SD. Percent suppression, shown in parentheses, was calculated as follows:

$$\left[1 - \left(\frac{\text{observed IgG in the presence of regulatory cells}}{\text{IgG secretion in the absence of regulatory cells}}\right)\right] \times 100$$

pressor-effector function [20]. More importantly, our data also suggest that the 2H4 molecule itself may be involved in generating suppressor signals [25]. In contrast, the T4+4B4+ subset of cells provides helper function in both pokeweed mitogen-stimulated IgG synthesis and antigen-specific antibody production assays [21,23]. Furthermore, T4+4B4+ cells, like T4+JRA− cells, proliferate well to soluble antigen stimulation but relatively poorly to autologous non-T cells [21,24].

The results demonstrating the scarcity of T4+2H4+ cells and substantial numbers of T4+4B4+ cells in synovial fluid in rheumatoid arthritis clarify in part some of the previously reported findings on synovial fluid lymphocyte function including the observation that synovial fluid lymphocytes respond poorly in the AMLR, although they may respond well to selected soluble antigens, and lack suppressor activity [12,13,15–17]. Moreover, the abundance of T4+4B4+ cells may explain the abundant production of immunoglobulin, including rheumatoid factors, and it is possible to speculate that the production of various lymphokines and other inflammatory mediators in the rheumatoid arthritic joint is promoted through related "helper" circuits. The findings of profound phenotypic and functional abnormalities in the synovial compartment as compared with minimally abnormal or normal findings in the peripheral blood are consistent with the previous observations of numerous investigators [1–18]. Two reports published since the completion of these studies also demonstrate in synovial fluid of patients with rheumatoid arthritis an increase in helper cells for antibody production and a decrease in the subpopulation containing suppressor-inducer cells [33,34]. One of these used a different panel of monoclonal antibodies, which we have previously shown [20] recognize different cell subpopulations than our reagents, and was limited to patients with rheumatoid

arthritis, healthy normal control subjects, and several patients with osteoarthritis [33]. The other study [34], which did use the monoclonal antibodies we developed, also was limited primarily to patients with rheumatoid arthritis, healthy control subjects, and patients with non-inflammatory arthritis (11 of 13 patients with other articular diseases studied). Several important points made by our study substantially extend these observations. First, the scarcity of T4+2H4+ cells and increased numbers of T4+4B4+ cells in the synovial fluid are not limited to the synovial fluid of patients with rheumatoid arthritis. Similar findings are also observed in synovial fluid of patients with inflammatory joint diseases including not only the seronegative arthropathies but also crystal-induced acute joint inflammation. Thus, these changes do not represent a pathologic change unique to rheumatoid arthritis or even chronic inflammatory joint disease, but occur as part of a more generalized phenomenon. Second, patients with disorders that may have a more systemic or diffuse inflammatory component (Reiter's, Sjögren's, scleroderma, rheumatoid arthritis with Felty's) can exhibit such alterations in their peripheral blood, in contrast to most patients with rheumatoid arthritis. Third, we have shown that these phenotypic changes have functional implications by use of in vitro assays that may reflect and explain some of the previously described observations made with rheumatoid arthritis synovial T cells. Finally, we have demonstrated in a preliminary fashion that a diminution in this important regulatory population of T4+2H4+ suppressor-inducer cells may occur in association with increased age.

The mechanisms responsible for the paucity of T4+2H4+ cells and the increased representation of T4+4B4+ cells are not clear at this time and may differ in the various disorders. One principal possibility is that synovial T cells may already be in an intensely activated state, as suggested by their considerably elevated expression of Ia and other activation antigens. Because the 4B4 antigen is expressed on both resting and activated T4 cells, while 2H4 antigen density eventually decreases after cell activation and is often eventually lost from activated cells [35, 36, and this study], the relative excess of 4B4+ cells in the joint may reflect, at least in part, their activation state. Thus, in conditions where lymphocytes are intensely activated such as synovial fluid, there may be more 4B4+ T cells and less 2H4+ T cells. In addition, our earlier studies showed that the density and expression of the 2H4 antigen were strongly correlated with suppressor-inducer function in both AMLR and concanavalin A systems [35,36]. Alternative mechanisms for this concentration of T4+4B4+ cells might be an increased responsiveness of these cells to chemoattractants present in inflammatory joint effusions or differential entry of particular subsets of T4 cells into the joint. With respect to the latter, Jalkanen et al [37] have shown that the specific receptors for T-cell adherence to synovial endothelial cells differ from those for adherence to mucosal or lymph node endothelial cells. Thus, particular T cell subsets may be programmed to enter the synovium. The consequent lack of T4+2H4+ cells might therefore be due to their relative inability to adhere to the endothelial cells of synovial blood vessels, whereas T4+4B4+ cells may represent the subset programmed to enter the joint.

In this regard, recent studies from our group have shown that the 4B4 molecule is comprised of 135-, 160-, and 185-kilodalton glycoproteins and belongs to a family of cell adhesion molecules that includes the fibronectin receptor and related structures [38]. Further studies are now in progress to determine whether T4+4B4+ cells preferentially adhere to the endothelial cells of the synovium. If this is the case, then in the presence of a variety of immunologic stimuli, there could be ongoing immunologic reactivity due to a lack of capacity for induction of suppression in association with an abundance of helper cells. This could promote the formation of immune complexes and the release of lymphokines and other inflammatory mediators as secondary and tertiary events in the evolution of rheumatoid as well as other forms of inflammatory synovitis. The mechanisms by which such a response is regulated are at this time unknown.

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