Response of immunoregulatory lymphocyte subsets to methotrexate in rheumatoid arthritis

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■ In an attempt to define the immunoregulatory mechanisms operating in rheumatoid arthritis, the authors examined peripheral blood functional lymphocyte subsets in 15 patients with active rheumatoid arthritis who were not receiving remittive therapy, as well as 33 healthy controls. The percentage and absolute numbers of total T cells (CD3), T-helper/inducer cells (CD4), and T-suppressor/cytotoxic cells (CD8) did not differ among the groups, nor did the CD4:CD8 ratio or the numbers of T cells coexpressing CD4 and the activation markers Ia or IL-2R. However, rheumatoid arthritis patients did have reduced percentages and numbers of $CD4^+$ cells coexpressing the 2H4 antigen (CD45R-naive T cells) ($P<.0003$) and $CD8^+$ cells coexpressing the Leu-15 (CD11b) marker (suppressor/effectors) ($P<.0005$). Twelve patients then received oral methotrexate, 7.5 mg weekly. Most showed clinical improvement by 4 weeks and all did by 8 weeks. Although changes in the T-cell subsets were not statistically significant, several tended toward normalization. These findings may help explain the immunoregulatory defect in rheumatoid arthritis and the effectiveness of methotrexate in modifying disease activity.

□INDEX TERMS: ARTHRITIS, RHEUMATOID; METHOTREXATE □CLEVE CLIN J MED 57:232–241

RHEUMATOID ARTHRITIS is characterized by a variety of defects of immunologic function. Although broad immunologic defects have been described, including humoral and B-cell abnormalities as well as natural killer and macrophage dysfunction, T-cell function has received significant attention. Investigators have attempted to define T-cell function using monoclonal antibodies directed against helper/inducer (CD4) and suppressor/cytotoxic (CD8) determinants, but their efforts have produced conflicting results.[1]

■ *See accompanying editorial by Olsen (pp 245-246)*

Recently, CD4 T lymphocytes have been separated into at least two functionally distinct subpopulations. CD4 cells expressing 2H4 (CD45R) have been designated suppressor/inducers because of their ability to induce CD8-bearing T cells to exert suppressor/effector function[2]; CD4 cells bearing 4B4 (CD29) have been designated helper/inducers because of their ability to

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TABLE 1
MONOCLONAL ANTIBODIES USED TO ENUMERATE T-CELL SUBSETS

Monoclonal antibody	Manufacturer	Cluster designation (CD#)	Description
Leu 2	Becton-Dickinson	CD8	Suppressor/cytotoxic
Leu 3	Becton-Dickinson	CD4	Helper/inducer
Leu 4	Becton-Dickinson	CD3	Matue peripheral-blood T cell
Leu 15	Becton-Dickinson	CD11b	Suppressor/effector when co-expressed with Leu-2 (ie, $Leu2^+15^+$)*
HLA-DR (Ia)	Becton-Dickinson	–	"Activation" marker for T cells
Interleukin-2 receptor (IL-2R)	Becton-Dickinson	–	"Activation" marker for T cells
2H4	Coulter	CD45R	Naive T cells, suppressor/inducer when co-expressed with Leu-3 (ie, $Leu3^+2H4^+$)
4B4	Coulter	CD29	Memory T cells, helper-inducer when co-expressed with Leu-3 (ie, $Leu3^+4B4^+$)

*The reciprocal $Leu\text{-}2^+15^-$ subset (CD28) defines cytotoxic effectors

help B cells in pokeweed mitogen–driven immunoglobulin production.[3]

More recent studies suggest that the 2H4-bearing suppressor/inducer cell populations and the 4B4-bearing helper/inducer cell populations are not distinct lineages but reflect different maturational stages of T-cell development.[4,5] Furthermore, these subsets can be more generally designated *naive* T cells (encompassing suppressor/inducers) and *memory* T cells (encompassing helper/inducers), characterized by differences in activation requirements and lymphokine secretion.[6]

We determined the numbers and percentages of these subpopulations in the peripheral blood of untreated rheumatoid arthritis patients with active disease and investigated the effects of low-dose oral methotrexate on these subsets and on the patients' clinical status.

METHODS

Clinical studies

The baseline study group comprised 15 patients (14 women, 1 man) with active rheumatoid arthritis, all of whom were receiving stable doses of nonsteroidal anti-inflammatory agents (including aspirin). Thirteen of the 15 patients were seropositive for rheumatoid factor at the time of the baseline blood draw. None of the patients was receiving or had received any disease-modifying drug or prednisone in doses >10 mg/d or intra-articular glucocorticoids within 60 days of entering the study. *Active disease* was defined as the presence of ≥5 tender joints (with or without swelling). All but one of the 15 had a Westergren sedimentation rate (WSR) >25 mm/h.

Baseline investigations included clinical data (number of tender joints, AM gel, grip strengths, complete blood count with differential, WSR, C-reactive protein, rheumatoid factor, C1q binding assay (C1qBA), SGOT); peripheral blood mononuclear cell (PBMC) subset analysis; and PBMC mitogen studies. Baseline values also were obtained for 33 healthy controls.

A second study group comprised 10 of the 15 above-mentioned patients and 2 other patients with active, untreated rheumatoid arthritis who met the same entry criteria. They began weekly, low-dose (7.5 mg) oral methotrexate administration when they entered the study. These 12 patients were re-evaluated at 4 and 8 weeks with the same tests used at baseline. We developed an index to assess percent improvement at 4 and 8 weeks. Percent change (positive or negative) in the number of tender joints, minutes of AM gel (maximum possible, 240 minutes), mean grip strength (right + left/2) and C-reactive protein (mg/dL) were calculated. The average of the best three of four percentages was the improvement index.

Enumeration of T-cell subsets

After Ficoll-Hypaque density gradient separation, PBMC were suspended at 2×10^6 to 10×10^6 cells/cc, and 100 µL of cell suspension was placed in 13 × 75-mm tubes. For single-marker analysis, 20 µL of conjugated monoclonal antibody was added to each tube, and, for dual-marker analysis, 20 µL of a second monoclonal antibody was added simultaneously (one monoclonal antibody was conjugated to fluorescein isothiocyanate [FITC] and one to phycoerythrin [PE]). Following incubation at 4°C for 30 minutes with vortexing every 10 minutes, the cells were washed and fixed in 0.5 cc 2% buffered paraformaldehyde. The monoclonal antibodies used are listed in *Table 1*, with a brief functional description and cell cluster designations, where designated.

Single- or dual-marker studies were conducted on a FACS 440 fluorescence activated cell sorter (Becton-

TABLE 2
CLINICAL FINDINGS IN RHEUMATOID ARTHRITIS PATIENTS AND CONTROLS

	Rheumatoid arthritis patients (n = 15)		
	Mean (±SD)	Range	Normal values
Age	46.9 (17.3)	26–69	–
Disease duration (yr)	4.46 (4.88)	0.38–>20	–
AM gel (min)	129 (73)	15–>240	–
Number of tender joints	21.5 (11.0)	5–41	0
Grip strength* (mmHg)			
R	126.5 (67.0)	70–260	>200
L	111.6 (70.6)	50–270	>200
Hemoglobin (g/dL)	12.1 (1.4)	9.6–14.0	12–16
White blood cells (cells/μL)	8,173(2,063)	5,400–13,700	4,000–11,000
Lymphocytes/mm³	1,812 (634)	800–3,500	1,000–4,000
Lymphocytes (%)	23.3 (9.6)	11.0–42.0	15–45
Platelets (k/μL)	403 (94)	282–577	150–400
SGOT† (IU/L)	28 (29)	10–111	7–40
Rheumatoid factor‡ (IU/mL)	712 (717)	142–>2,440	0
C1qBA (U/mL)	467 (821)	25–2,783	<73
WSR (mm/h)	54 (28)	23–117	<20
C-reactive protein (mg/dL)	4.1 (4.4)	0.6–15.0	<2.0

*n = 8
†n = 12
‡13 of 15 patients were seropositive at the time of the initial blood draw

Dickinson, FACS Division, Sunnyvale, CA) equipped with an argon laser (200 mW at 488 nm) used for excitation of FITC or PE. Fluorescence emission for FITC and PE was selectively detected by collecting light at 530 ± 15 nm (for FITC) and 575 ±26 nm (for PE). Forward and 90° angle light scatter, green fluorescence (FITC), and orange fluorescence (PE) were measured and stored for reanalysis by a Consort 40 PDP 11/73 computer system (Becton Dickinson, FACS division). In single-marker analysis, the percentage of positive cells was determined after computer subtraction of background fluorescence for a particular cell-surface marker. For dual-parameter analysis, a computer program was used that allows analysis of a second marker in a certain subset, or *slice*, of cells analyzed for the first marker.

For control, 20 μL of mouse IgG1, IgG2, or IgM, conjugated to FITC or PE, was added to a similar aliquot of cells. Absolute numbers of cells were determined by multiplying the percent of the cell of interest times the absolute number of lymphocytes derived from a simultaneously drawn complete blood count with differential.

Mitogen and antigen proliferation studies

Lymphocyte transformation testing with mitogens and antigens was carried out as described by Malnish and Strong[7] using Ficoll-Hypaque–separated PBMC cultured for 96 hours in RPMI-1640 supplemented with 10% human AB serum (Grand Island Biological, Grand Island, NY). Tritiated thymidine (1 μCi) was included during the last 20 hours of incubation. Optimal doses of the following T- and/or B-cell mitogens were tested, alone or in combination: phytohemagglutinin (DIFCO, Detroit, Mich), pokeweed mitogen (GIBCO, Grand Island, NY), concanavalin-A (Miles-Yeda, Rehovot, Israel), and Cowan strain *Staphylococcus aureus* (Bethesda Research Laboratories, Gaithersburg, Md), as well as the soluble antigens, tetanus toxoid (Wyeth, Marietta, Pa), and *Candida* (Hollister-Steir, Atlanta, Ga). Responses were expressed as counts per minute of tritiated thymidine incorporation per 2×10^5 cells. Both intra- and interassay variables for this test range from 15% to 20%.

Statistical methods

Analyses of variance and Student's *t* tests were used to compare T-cell subsets. Results at baseline and at 4 and 8 weeks of treatment were compared using repeated-measures ANOVA. Spearman rank correlation coefficient was used to assess the correlation between clinical features and T-cell subsets. Ninety-five percent confidence limits were determined for mean differences. Categorical data were analyzed using Fisher's Exact test or Chi-square, depending on expected cell frequencies. SAS[8] software was used to perform all statistical tests and data management.

TABLE 3
PERCENTAGES, TOTALS, AND RATIOS OF SELECTED T-CELL SUBSETS IN RHEUMATOID ARTHRITIS PATIENTS AND CONTROLS

	Patients (n = 15)		Controls (n = 33)		
	Mean	(± SD)	Mean	(± SD)	P-value
T-cell subset					
Leu-4$^+$					
%	71.9	(8.5)	74.3	(8.1)	.40
Cells/mm^3	1,338	(576)	1,364	(316)	.87
Leu-4$^+$Ia$^+$					
%	4.8	(2.8)	4.8	(2.0)	1.00
Cells/mm^3	76	(31)	89	(40)	.29
Leu-4$^+$IL-2R$^+$					
%	1.9	(1.4)	2.0	(0.9)	.80
Cells/mm^3	30	(24)	37	(18)	.31
Leu-3$^+$					
%	48	(11)	50	(6.9)	.50
Cells/mm^3	895	(414)	915	(239)	.86
Leu-3$^+$2H4$^+$					
%	13.1	(6.2)	26.9	(6.8)	.0001
Cells/mm^3	243	(142)	483	(183)	.0002
Leu-3$^+$4B4$^+$					
%	28.0	(11.2)	21.0	(5.5)	.01
Cells/mm^3	556	(329)	385	(131)	.04
Leu-2$^+$					
%	25.7	(5.6)	28.4	(6.6)	.21
Cells/mm^3	458	(178)	524	(179)	.29
Leu-2$^+$15$^+$					
%	5.8	(3.5)	11.4	(4.8)	.0005
Cells/mm^3	98	(56)	214	(121)	.002
Leu-2$^+$15$^-$					
%	20.0	(5.8)	17.2	(4.4)	.11
Cells/mm^3	340	(191)	315	(95)	.61
Ratio					
Leu-3:Leu-2	2.0	(0.7)	1.9	(0.7)	.68
Leu-3$^+$4B4$^+$:Leu-3$^+$2H4$^+$	2.71	(1.66)	0.82	(0.31)	.0006
Leu-2$^+$15$^-$:Leu-2$^+$15$^+$	4.84	(3.27)	2.06	(1.41)	.003

RESULTS

Baseline study

Clinical characteristics. The clinical and laboratory findings of the 15 arthritis patients are outlined and compared with normal values in *Table 2*.

T-cell subsets. Results of dual-labeling studies for untreated rheumatoid arthritis patients with active disease and controls are shown in *Table 3*. There were no differences in the percentages or numbers of total circulating mature T cells (Leu-4), helper/inducer T cells (Leu-3), or suppressor/cytotoxic T cells (Leu-2), nor any difference in the Leu-3:Leu-2 ratio. Similarly, no differences were seen between patients and controls in percentages or numbers of T cells bearing the HLA-DR or IL-2R activation markers.

There were significant differences in percentages and numbers of both the Leu-3$^+$2H4$^+$-naive subset and the Leu-2$^+$15$^+$ suppressor/effector (SE) subset. The percentages and numbers of Leu-3$^+$4B4$^+$ memory T cells tended to be higher in the rheumatoid arthritis patients, but this tendency did not reach statistical significance (at a P <.01 level of significance).

Figure 1 shows the distribution of percentages of Leu-3$^+$2H4$^+$ cells in active rheumatoid arthritis patients v controls. The mean percentage of naive T cells in the peripheral blood of controls was 26.9%. If we consider any value less than two standard deviations below the mean for controls (ie, <11.1%) to be significantly lower than normal, then 7 of 15 patients with active rheumatoid arthritis and none of the 28 controls had a deficiency of circulating Leu-3$^+$2H4$^+$ cells (P = .0002, by Fisher's Exact test).

As noted above, there was no difference between patients and controls in the ratio of Leu-3$^+$ to Leu-2$^+$ cells. There was a significant difference, however, in the Leu-3$^+$4B4$^+$:Leu-3$^+$2H4$^+$ ratio between patients and controls (P = .0006). *Figure 2* shows the distribution of the

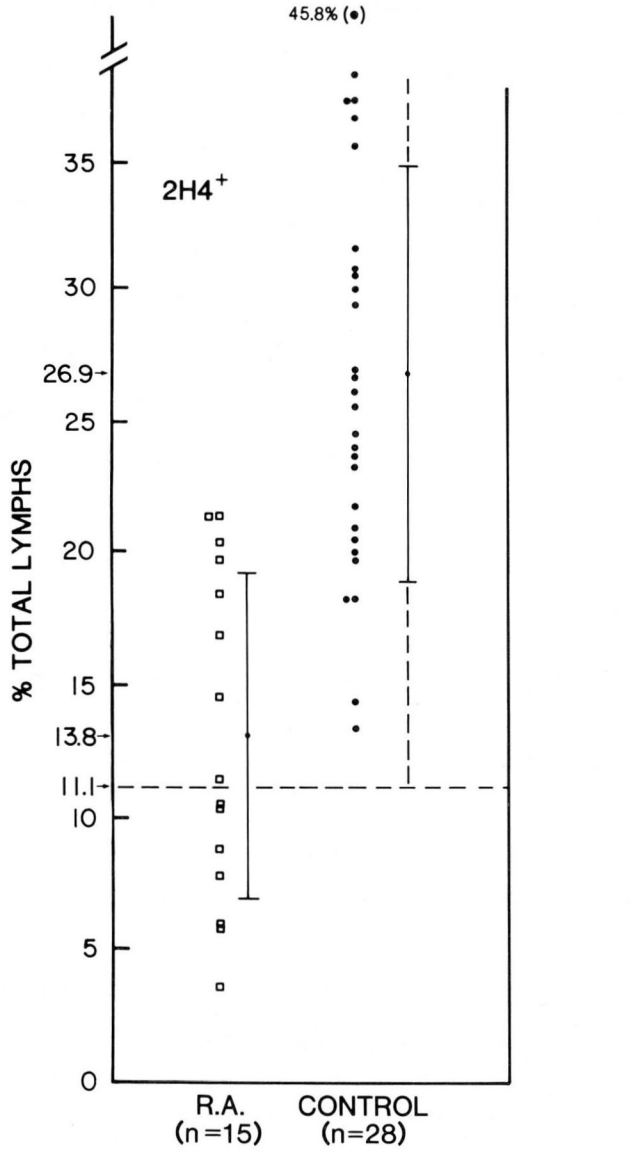

FIGURE 1. Distribution of percentages of Leu-3+2H4+ (suppressor/inducer) cells for patients and controls. The dashed line (at 11.1%) is 2 SD below the mean for normals. Seven of 15 rheumatoid arthritis patients, but none of 28 controls fall below this line ($P = .0002$).

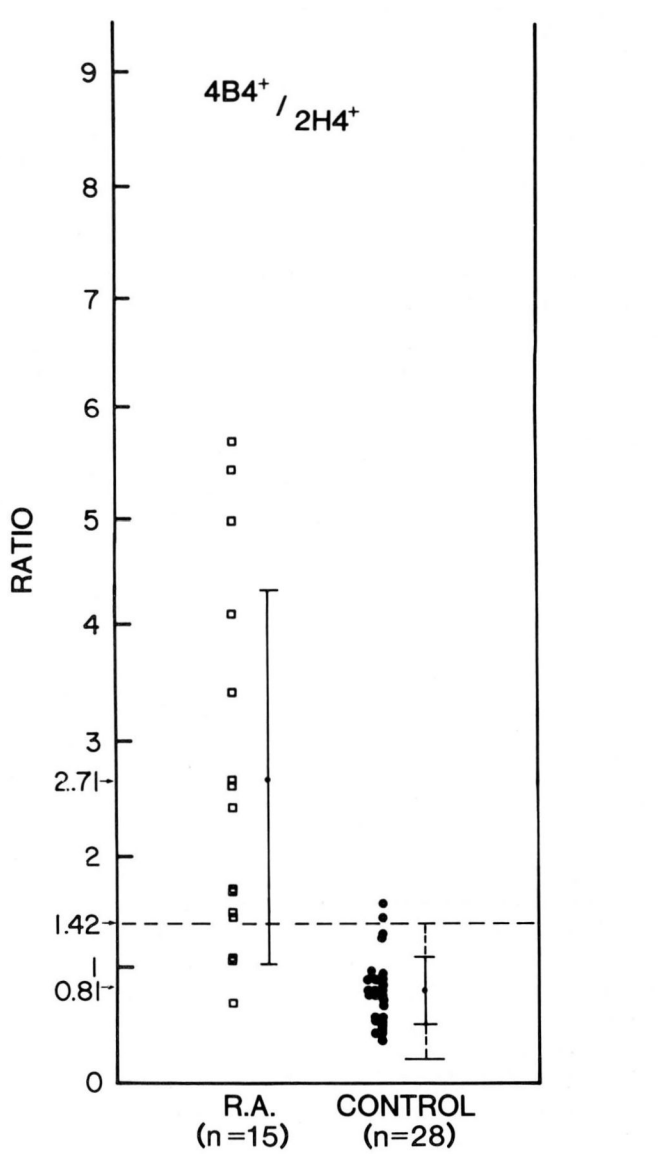

FIGURE 2. Distribution of ratios of (Leu-3+) 4B4+:2H4+ (helper/inducer:suppressor/inducer) cells for patients and controls. The dashed line (at 1.42%) is 2 SD above the mean for normals. Twelve of 15 rheumatoid arthritis patients, but only 2 of 28 controls lay above this line ($P = .000002$).

ratios of memory to naive T cells in patients with active rheumatoid arthritis and controls. Again, if we consider any value greater than two standard deviations above the mean for controls (ie, >1.42) to be significantly higher than normal, 12 of 15 patients but only two of 28 controls had elevated ratios ($P = .000002$, by Fisher's Exact test).

No statistically significant correlation was found between the relative frequency of any of the T-cell subsets and any of the objective measures of disease activity

TABLE 4
CLINICAL FINDINGS IN 12 RHEUMATOID ARTHRITIS PATIENTS AT BASELINE AND AT 4 AND 8 WEEKS OF TREATMENT WITH LOW-DOSE METHOTREXATE

	Mean (± SD)			P-values for interval change	
	Baseline	4 wk	8 wk	0–4 wk	0–8 wk
Number of tender joints	24.7 (8.9)	19.1 (10.1)	13.8 (6.0)	.005	.003
AM gel (min)	151 (77)	102 (71)*	75 (57)	.075	.010
Grip strength (mmHg)					
R	98 (42)†	123 (47)	135 (44)	.035	.018
L	84 (30)†	119 (57)	121 (45)	.013	.014
Hemoglobin (g/dL)	12.1 (1.4)	11.8 (1.6)	11.9 (1.6)	.581	.624
White blood cells (cells/μL)	7,850 (1,611)	8,217 (3,022)	8,225 (2,894)	.638	.633
Lymphocytes/mm³	1,938 (787)	1,604 (491)	1,466 (587)	.099	.045
Lymphocytes %	25.2 (10.0)	22.3 (11.1)	19.0 (9.6)	.366	.056
Plat (k/μL)	413 (77)	391 (96)	398 (80)	.255	.108
Rheumatoid factor (IU/mL)	637 (743)	655 (877)	670 (888)	.103	.154
C1q (U/mL)	431 (830)	202 (388)‡	131 (213)	.011	.011
WSR (mm/h)	50.6 (23.1)	40.5 (21.5)	39.3 (14.7)	.025	.011
C-reactive protein (mg/dL)	4.1 (3.3)	3.0 (2.8)	2.5 (2.2)	.024	.014

*n = 11
†n = 9
‡n = 10

studied, including number of swollen joints, grip strength, AM gel, WSR, C-reactive protein, rheumatoid factor, or C1q.

Mitogen and antigen proliferation studies. No significant differences were noted between patients with active untreated rheumatoid arthritis and healthy controls in proliferative response of PBMCs to phytohemagglutinin, pokeweed mitogen, concanavalin-A, or Cowan strain *Staphylococcus aureus*. In studies using the soluble antigen, tetanus toxoid, patients with rheumatoid arthritis were less likely to show a proliferative response (stimulation index >2; 13/23 controls, 2/11 rheumatoid arthritis patients, $P < .05$). There was, however, no correlation between in vitro anergy of PBMCs to the tetanus toxoid and the percentage or number of any of the T-cell subsets studied.

Methotrexate study

Clinical and laboratory evaluation. All 12 patients who received methotrexate showed some clinical improvement. At 4 weeks, the patients showed a 28.3% mean improvement in their index; at 8 weeks, mean improvement was 45.3%. Individual improvement by 8 weeks was distributed as follows: five patients showed <30% improvement, one showed 30% to 50% improvement, and six showed >50% improvement in their indices.

Table 4 shows the changes in clinical and laboratory parameters from baseline to 4 weeks and 8 weeks. P-values <.02 were considered significant. Significant improvement was seen at 8 weeks in the number of tender joints, AM gel, grip strength, C1q, WSR, and C-reactive protein. Although there was a trend toward continued improvement in the number of tender joints ($P = .045$) and AM gel ($P = .076$) between 4 and 8 weeks, there were no statistically significant differences between 4 and 8 weeks for any variable. As *Table 4* shows, at 4 weeks there was already a definite trend toward improvement, if not a significant difference, in all measures at 8 weeks. Side effects of the medication were infrequent and mild (one case of first-dose nausea) and were not cause for discontinuing methotrexate.

T-cell subsets. *Table 5* shows the results of dual-labeling studies for the 12 patients treated with low-dose methotrexate. At 8 weeks, there were no significant changes noted in any subset (at a $P < .02$ level of significance). However, apparent trends were noted in several subsets over the 8 weeks of treatment, including decreasing numbers of total Leu-4 and Leu-2 cells. The decrease in the former subset reflects the trend toward decreased numbers of total lymphocytes (see *Table 4*). The decrease in the latter (Leu-2) subset reflects a decrease in the Leu-2^+15^- cytotoxic effector subset rather than the Leu-2^+15^+ suppressor/effector subset, which actually showed a trend toward increasing percentage. There was also a trend toward increasing percentage of the Leu-3^+2H4^+ suppressor/inducer subset (*Figure 3*). No significant differences were found in T-cell subset percentages at 8 weeks between patients who showed <30% im-

TABLE 5
PERCENTAGES, TOTALS, AND RATIOS FOR SELECTED T-CELL SUBSETS AT BASELINE AND AT 4 AND 8 WEEKS OF TREATMENT WITH LOW-DOSE METHOTREXATE

	Mean (± SD)			P-values for interval change	
	Baseline (n = 12)	4 wk (n = 12)	8 wk (n = 11)	0–4 wk	0–8 wk
T-cell subset					
Leu-4					
%	72.6 (9.6)	70.4 (9.3)	70.1 (13.4)	.479	.563
Total	1,461 (727)	1,159 (464)	1,094 (569)	.092	.068
Leu-4$^+$Ia^{+*}					
%	4.6 (3.0)	3.3 (1.1)	3.2 (1.5)	.091	.284
Total	74 (30)	49 (14)	46 (22)	.041	.045
Leu-3					
%	46.0 (10.0)	46.9 (9.8)	46.0 (10.3)	.610	.505
Total	938 (490)	784 (355)	733 (422)	.170	.142
Leu-3$^+$2H4$^+$					
%	12.6 (6.6)	15.4 (8.4)	17.9 (9.8)	.255	.068
Total	248 (161)	250 (167)	283 (219)	.965	.563
Leu-3$^+$4B4$^+$					
%	24.1 (7.7)	22.6 (7.3)	21.5 (7.4)	.182	.505
Total	446 (206)	381 (191)	345 (220)	.120	.103
Leu-2					
%	26.1 (3.5)	26.0 (5.8)	26.6 (5.5)	.812	.937
Total	495 (194)	410 (126)	381 (144)	.055	.029
Leu-2$^+$15$^+$					
%	5.4 (4.0)	7.1 (4.5)	7.8 (6.7)	.045	.056
Total	92 (58)	109 (71)	98 (59)	.262	.505
Leu-2$^+$15$^-$					
%	20.4 (4.8)	18.0 (3.5)	18.3 (4.7)	.170	.182
Total	369 (217)	288 (107)	276 (135)	.092	.075
Ratio					
(Leu-3) 4B4$^+$:2H4$^+$	2.5 (1.4)	2.1 (1.6)	1.9 (1.8)	.266	.350
(Leu-2) 15$^-$:15$^+$	5.7 (3.5)	3.6 (2.0)	3.8 (2.5)	.017	.083

provement and those who showed >50% improvement in their clinical improvement index.

Mitogen-antigen proliferation studies. There were no differences in lymphocyte responsiveness to mitogens after 4 or 8 weeks of methotrexate administration.

DISCUSSION

Previous studies have failed to identify clear associations between CD4 helper and CD8 suppressor populations and lymphocytic function; this is consistent with the evidence that these populations are highly heterogeneous. The ability to identify phenotypically and functionally distinct subpopulations of T cells using dual-marker analysis has broadened our understanding of the immunoregulatory events operating in a number of immunologic conditions.

Changing concepts of T-cell subpopulations

A series of newly developed monoclonal antibodies has allowed us to divide the CD4 T-helper cell population into numerous functional or immunoregulatory subsets. Separating CD4 cells based on their coexpression of 2H4 (CD45R) or 4B4 (CD29) was originally thought to define two distinct populations of T cells with either suppressor/inducer or helper/inducer functions based on their in vitro characteristics.[2,3]

More recent investigations suggest that these subpopulations represent a dynamic continuum reflecting different maturational stages of T cells.[4–6] CD4-bearing T cells generally coexpress 2H4 (CD45R) and 4B4 (CD29) determinants, although the density of their expression is reciprocal (ie, high 2H4 expression, low 4B4 expression or high 4B4 expression, low 2H4 expression). T cells coexpressing CD4 and 2H4 (CD45R) represent naive T cells—they do not proliferate in response to antigen in vitro. Indeed, these cells encompass T lymphocytes exerting suppressor/inducer function, but these functional characteristics are not static. T helper cells bearing 2H4 can be induced to decrease their expression of 2H4 and increase their expression of 4B4 in response to antigen or mitogen.

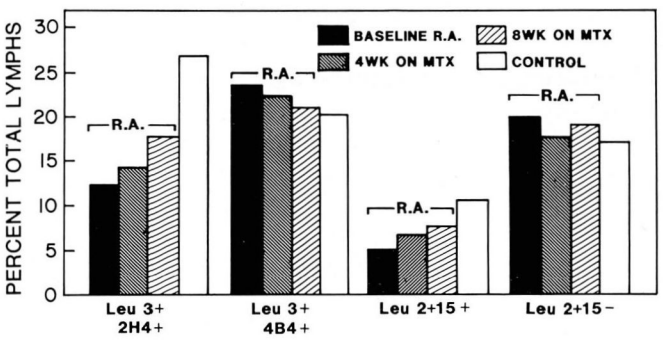

FIGURE 3. Graph display of percentages of selected T-cell subsets at baseline, 4 weeks, and 8 weeks for rheumatoid arthritis patients and controls (Table 2).

TABLE 6
DIFFERENCES BETWEEN NAIVE AND MEMORY T CELLS[6]

	Naive	Memory
Proliferation to:		
Recall antigens	±	++++
Allogeneic cells	++++	++++
Autologous cells	++++	+
Phytohemagglutinin	++++	++
Concanavalin A	++++	++
Lymphokine secretion:		
IL-2	++++	++++
IL-3	+	++++
IFN-γ	±	++++
Modulation of B-cell responses:		
Help for Ig production	±	++++
Suppression of:		
Polyclonal Ig production	++++	±
Specific Ig production	±	++++

T cells expressing a high density of 4B4 antigen are considered to have memory function, since they proliferate in response to specific antigen. This latter subpopulation of cells encompasses T helper cells with helper/inducer function for B cells in response to pokeweed mitogen. Thus, the formally recognized helper/inducer and suppressor/inducer T-cell subpopulations are a dynamic continuum when influenced by appropriate stimuli. The differences among these cell populations are summarized in *Table 6*.

Recent studies have also demonstrated that several other antigens also share similar relationships with naive and memory T cells. The antigenic determinants LFA-3 and UCHL 1 are also found in low density on naive T cells (2H4 high) and increase their expression dramatically in response to appropriate activating stimuli and subsequent transformation to memory T cells.[9] Memory and naive T-cell populations also differ in their ability to secrete lymphokines and respond to other mitogenic stimuli such as anti-CD3 and autologous cells.[6] The concept that these cell populations represent naive and memory cells rather than purely suppressor/inducer and helper/inducer subpopulations has allowed us to reinterpret reports of abnormal distributions of these cells in a variety of immunologic conditions.

The CD8 or cytotoxic/suppressor population of T cells also coexpresses 2H4 CD45R and 4B4 (CD29) antigens. The relationships between the expression of these antigens on CD8-bearing cells and naive and memory status has not been as well studied as in the CD4-bearing population, although there is growing evidence of a similar dynamic relationship.[10] The antigenic determinant identified by the monoclonal antibody Leu-15 (CD11b) has been shown to react with a variety of cell types including the subset of CD8-bearing T cells. This subset has been shown by Clement and associates [11] to suppress in vitro T-cell proliferation responses to soluble antigens or mitogens as well as pokeweed mitogen–stimulated B cell immunoglobulin production. These cells have been designated suppressor/effectors. The $CD8^+$ Leu-15 (CD11b)–negative subset has been shown to comprise precursor and effector cytotoxic T cells and demonstrate virtually no suppressor activity.[12]

T-cell subsets in rheumatoid arthritis

We have clearly identified several abnormalities in the numbers and percentages of immunoregulatory T cell subsets in patients with active untreated rheumatoid arthritis. Rheumatoid arthritis patients demonstrated a depressed number and percentage of naive T cells $CD4^+2H4^+$ (CD45R) in peripheral blood compared with healthy controls. While the lack of an age-matched control population could influence these data, the fact that 7 of 15 *v* 0 of 28 controls fell below two standard deviations of the mean for controls reflects the magnitude of the deficit. In addition, we identified a deficit of $CD8^+$Leu-15^+–bearing suppressor/effector cells in this patient group. While other cell populations demonstrated interesting trends, such as the increase in $CD4^+4B4^+$ (CD29) memory T cells, the changes did not reach statistical significance.

Other investigators have examined the $CD4^+2H4$ (CD45R) cell population in rheumatoid arthritis,[13,14] but only one has demonstrated findings similar to ours.[15] We believe this reflects the marked heterogeneity among the rheumatoid arthritis subjects in these studies

with regard to treatment and disease activity. In view of these differences, we believe that this cellular deficiency may reflect disease activity.

We also identified a deficit in the $CD8^+Leu\text{-}15^+$ (CD11b) suppressor/effector cell population. This defect has also been identified by Goto and associates[16] and may reflect a deficit of cellular suppression. A number of functionally based lymphocytic studies support this finding.[11,17]

In contrast to studies of peripheral blood, numerous studies of immunoregulatory T-cell subsets in synovial fluid and tissues have consistently demonstrated deficits of the $CD4^+2H4$ (CD45R)–naive T-cell population and an increase in the $CD4^+4B4^+$ (CD29) memory T-cell population in rheumatoid arthritis.[13,14,18] In addition, similar findings have been reported in synovial tissues of patients with other inflammatory rheumatic disease, including psoriatic arthritis, gout, and spondyloarthropathy.[13] The reasons for the discrepancies between synovial fluid and peripheral blood are unclear.

The significance of these changes in T-cell subpopulations is not known, but is open to several interpretations. If these populations are viewed as suppressor/inducers and helper/inducers, these results could explain some of the basic immune derangements in rheumatoid arthritis, such as decreased suppressor-cell activity and decreased ability of T cells from rheumatoid arthritis patients to respond to such stimuli as autologous cells and concanavalin-A.[19]

Alternatively, if these cell populations are viewed as primarily naive and memory cells, the changes in populations may merely reflect enhanced conversion of naive T cells to memory T cells in an ongoing immune process rather than reflecting a primary immune dysregulatory event. Similar defects in these cell populations have been identified in other immune conditions such as multiple sclerosis,[20] systemic lupus erythematosus,[21] and tuberculosis.[22]

The etiology of these deficiencies is unknown, but several have been proposed. One possibility is that there are specific antilymphocyte antibodies against these subsets. Such antibodies reactive with $CD4^+2H4^+$ (CD45R) cells have recently been identified in patients with systemic lupus erythematosus,[23] and antilymphocyte antibodies have been identified in patients with rheumatoid arthritis.[24]

Alternatively, these cumulative deficits may correlate with disease activity, since this finding has not been universal in rheumatoid arthritis subjects who were not selected on the basis uniform disease activity.

A genetic factor is possible, although Morimoto and associates[21] found no deficiency in the $2H4^+$ subset in healthy family members of patients with multiple sclerosis.

Methotrexate—an immunologic mechanism of action?

Methotrexate is a safe and effective treatment for rheumatoid arthritis.[25] While much is known of its effects on cancer cells at high doses, little is known of its effects on the immune system in low doses (7.5 mg to 15 mg per week) used to treat rheumatoid arthritis. Because of its unusually rapid onset of action and rapid loss of benefit when discontinued, compared with other agents aimed at inducing remission of rheumatoid arthritis, many believe methotrexate to be primarily anti-inflammatory, not immunomodulatory.

Our study further confirms the clinical efficacy and early onset of action of methotrexate in patients with active rheumatoid arthritis. In addition, we have attempted to define the mechanism of action by focusing primarily on the T-cell limb of the immune system. Although we detected no significant changes in any immunoregulatory T-cell subset during the study, we noted several trends.

Humoral parameters, including the erythrocyte sedimetation rate, C-reactive protein, and circulating immune complexes detected by C1q binding, all decreased during the 8-week methotrexate study period. Of greatest interest was the stepwise increase in naive T-suppressor cells (CD45R) and stepwise decline in memory T-helper cells (CD29), both toward normal levels. In addition, the depressed levels of T-suppressor/effector cells (CD11b negative) tended to normalize in the stepwise fashion during the study. Unfortunately, these trends did not achieve statistical significance, but it is possible that a more lengthy follow-up would have yielded different results.

If indeed the abnormalities in these subsets reflected a basic level of immune dysregulation in rheumatoid arthritis, a trend toward normalcy may reflect some basic action of the drug. Since clinical improvement comes chronologically earlier than changes in T-cell subsets, the trend may merely reflect a lessening of disease activity.

Other investigators have failed to identify changes in single-marker analysis of T-cell subsets including CD3, CD4, CD8, or CD4:CD8 ratios during low-dose methotrexate therapy.[26] Recent reports have identified several potential immunologic effects of the drug, including decreased in vitro production of immunoglobulin and polyclonal rheumatoid factor,[27] inhibition of cellular proliferation,[28] and decreased interleukin-1 activity,[29] all suggesting that low-dose methotrexate may

act more subtly as an immunosuppressive than other traditional immunomodulator agents.

SUMMARY

We have confirmed that in patients with active, untreated rheumatoid arthritis, there is a deficiency of naive T cells and an increase in memory T cells as well as a decrease in T suppressor/effector cells. Furthermore, we noted a stepwise trend toward normal of each of these subsets after 4 and 8 weeks of low-dose methotrexate therapy. We are unable to state whether these trends reflect a limited period of observation or merely represent epiphenomena, but speculate that these changes may indicate an immunologic mechanism of action of low-dose methotrexate.

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