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Clonal dominance among T-lymphocyte infiltrates in arthritis

(T-cell receptor/gene rearrangement/inflammation/blot hybridization)

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ABSTRACT **Synovial membranes in patients with rheumatoid arthritis as well as other types of chronic destructive inflammatory arthritis contain infiltrates of activated T lymphocytes that probably contribute to the pathogenesis of the disease. In an effort to elucidate the nature of these infiltrates, interleukin 2 (IL-2)-responsive T lymphocytes were grown out of synovial fragments from 14 patients undergoing surgery for advanced destructive inflammatory joint disease. Eleven of the samples examined were from patients with classical rheumatoid arthritis, while three others were obtained from individuals with clinical osteoarthritis. Southern blot analysis of T-cell receptor (TCR) β-chain genes in 13 of 14 cultures showed distinct rearrangements, indicating that each culture was characterized by the predominance of a limited number of clones. T-cell populations from peripheral blood stimulated with a variety of activators and expanded with IL-2 did not demonstrate evidence of similar clonality in long-term culture. These results suggest that a limited number of activated T-cell clones predominate at the site of tissue injury in rheumatoid synovial membranes as well as in other types of destructive inflammatory joint disease. Further characterization of these T-cell clones may aid our understanding of the pathogenesis of these rheumatic disorders.**

Rheumatoid arthritis is characterized by a chronic inflammatory process primarily involving the synovial membrane of peripheral diarthrodial joints. The synovitis is frequently accompanied by extraarticular manifestations and is associated with cellular and humoral immunological abnormalities (1, 2). Recent studies have suggested that the anti-IgG autoantibodies (rheumatoid factors), which are found in high frequency in classical rheumatoid arthritis, have a limited sequence diversity (3). Rheumatoid synovial membranes are characterized histologically by proliferating synovial lining cells and infiltrates of activated T cells, predominantly of the $CD4^+$ phenotype (4–6). Although it is recognized that these cells play an important role in the local inflammatory process (7, 8), their specificity and function are unknown. Activated T lymphocytes express receptors for interleukin 2 (IL-2) and can be propagated in culture medium containing this lymphokine (9). T cells propagated in this way conserve their specificity and function (10). In several studies, IL-2 has been used to selectively expand populations of T lymphocytes activated *in vivo* at the inflammatory sites of autoimmune diseases (11, 12), neoplasms (13), and organ rejections (14). These expanded populations have shown specificities relevant to the pathogenesis of the underlying disorder, such as proliferation in response to autologous thyroid epithelial cells in autoimmune thyroiditis (11), proliferation and cytotoxicity against alloantigens in transplanted organs (14), and cytotoxicity against autologous tumor (13).

The T-cell receptor (TCR) responsible for T-lymphocyte recognition of foreign antigens in association with molecules encoded by the major histocompatibility complex (MHC) is a disulfide-linked 90-kDa heterodimer consisting of two polypeptide chains, α and β, expressed on the T-cell surface (15–17). The β-chain genomic sequences of the TCR are rearranged in functional T-cell clones (18). The detection of such rearrangements has been used to determine the clonality of T lymphoproliferative disorders (18–20). Like the immunoglobulin genes, the *TCR* genes consist of noncontinuous variable (V), joining (J), diversity (D), and constant (C) region segments (21–24). The β-chain locus contains two constant regions ($C_{\beta1}$ and $C_{\beta2}$), each capable of rearrangement. Rearrangement of the β-chain gene complex can be detected by DNA blot hybridization using a cDNA constant region (C_β) probe. Hybridization of endonuclease-digested DNA with a constant region (C_β) probe detects rearrangements to either $C_{\beta1}$ or $C_{\beta2}$ depending on which endonuclease is used (18, 20, 24). With this approach, it is possible to detect the presence of a clone that constitutes 5% or more of the total population of cells analyzed as assessed by our own reconstruction experiments (data not shown) and by others (19). We therefore have attempted to expand the T-cell population of synovial specimens from patients with inflammatory joint disease in the presence of IL-2 and to analyze the TCR β-chain rearrangements in these cells and thereby assess the possible *in vivo* selection of a limited number of T-cell specificities at sites of inflammation.

METHODS

Clinical Specimens, Phenotypes, and Tissue Typing. The clinical diagnosis of rheumatoid arthritis or osteoarthritis was established from clinical records. Samples of synovial tissue were taken at surgery and at the initiation of cell culture for histological evaluation. Specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. The phenotype of the proliferating lymphocytes was established by analysis of cell-surface antigens (14). The monoclonal antibodies used were OKT3 ("pan" mature T cells), OKT4 (helper/inducer cells), OKT8 (cytotoxic/suppressor cells), and OKla-1 (HLA-DR constant region) (Ortho Diagnostics). The cells were typed according to standard microlymphocytotoxicity methods.

Culture of Synovial Tissue Lymphocytes. Synovial tissue was placed in RPMI medium supplemented with recombinant IL-2 (10 units/ml) (a generous gift from Sandoz Re-

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Abbreviations: IL-2, interleukin 2; TCR, T-cell receptor; MHC, major histocompatibility complex; V, variable; J, joining; D, diversity; C, constant; PHA, phytohemagglutinin; APC, antigen-presenting cell; MLR, mixed lymphocyte reaction.

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search, Vienna, Austria). T lymphocytes were grown out of each synovial sample for 10–15 days in the absence of lectin and then separated from the tissue of origin and expanded with phytohemagglutinin (PHA) (1 μg/ml) (Wellcome) in the presence of irradiated allogeneic mononuclear cells as described (14). Cells were harvested for DNA extraction prior to restimulation whenever their number reached 2×10^7 (10–28 days) and subsequently at monthly intervals for 2–6 months.

Culture of Peripheral Blood Lymphocytes. In control experiment 1, mononuclear cells isolated by Ficoll/Hypaque density centrifugation from the peripheral blood of five healthy individuals were cultured in RPMI medium containing 5% pooled heat-inactivated human serum with gentamycin (50 μg/ml). Cultures were established under five different conditions: (*i*) 15×10^7 cells in RPMI medium only; (*ii*) 15×10^7 cells in RPMI medium supplemented in IL-2 (10 units/ml); (*iii*) 10×10^7 cells with PHA (1 μg/ml); (*iv*) mixed lymphocyte reaction (MLR): 10×10^7 responder cells with 10×10^7 irradiated (3600 rad; 1 rad = 0.01 Gy) stimulator cells from unrelated donor; (*v*) 15×10^7 cells were stimulated with tetanus toxoid (1 μg/ml) and unrelated antigen-presenting cells (APCs). On day 3 after the initial stimulation, one-half of the cells were removed from the PHA cultures, and the lymphoblast fraction was isolated on a 50% Percoll gradient (density, 1.068) (25). These cells were grown in parallel with the remaining non-Percoll-separated cultures in RPMI medium and recombinant IL-2 (10 units/ml) at a starting concentration of 2×10^5 cells per ml. On day 5, the same procedure was applied to the MLR and tetanus toxoid cultures ($3–5 \times 10^7$ cells). All cell cultures were supplemented with fresh IL-2-containing medium twice a week. On days 14, 28, and 42, between 8 and 50×10^6 cells were removed from single cultures for DNA extraction.

In control experiment 2, blood (60 ml) was drawn from five healthy individuals and the mononuclear cell fraction was separated on Ficoll/Hypaque gradients and resuspended at a concentration of 1×10^6 cells per ml in RPMI medium supplemented in 5% pooled human serum (heat inactivated). For each individual, three initial cultures were established and the lymphocytes were stimulated with PHA, alloantigens (MLRs), and tetanus toxoid. The PHA cultures were supplemented with IL-2 on day 3 and diluted to 2×10^5 cells per ml, while the MLR and tetanus toxoid cultures were supplemented with IL-2 on day 5. The cultures were fed with fresh IL-2-containing medium twice a week and the cell density was never allowed to exceed 1×10^6 cells per ml.

On day 14, each of the PHA-, MLR-, and tetanus toxoid-induced cultures was divided into three cultures, one of which was not restimulated, whereas the other two were restimulated under two different conditions. Between 3 and 8×10^6 cells were recovered from the initial cultures and restimulated with the same antigen or mitogen used initially together with autologous fresh irradiated mononuclear APCs. The responder/feeder ratio ranged from 1:5 to 1:10. In parallel to the above cultures, a similar fraction of each of the initial PHA, MLR, and tetanus toxoid cultures was restimulated with a 5- to 10-fold excess of APCs from unrelated donors (buffy coats) and PHA (1 μg/ml). The cells were harvested for DNA extraction on days 28, 42, 56, and, in the case of 15 cultures, on day 90.

DNA Extraction and Southern Blot Analysis. DNA was prepared from freshly harvested cells after lysis with 3% lauryl sarcosinate and 3 mM EDTA, followed by proteinase K digestion, phenol extraction, and ethanol precipitation. DNA blots were done by the method of Reed and Mann (26).

Probes. The TCR β probe used was derived from a human cDNA clone Jurβ1 isolated from the Jurkat T lymphoma cell line and generously provided by Tak W. Mak (The Ontario Cancer Institute, University of Toronto, ON, Canada). The probe was inserted into an M13 origin PMLC12 plasmid (B. Seed, personal communication) and single-stranded DNA was prepared according to described procedures (27). To obtain the C region only, the Jurβ1 probe was cut with *Pst* I and *Bgl* II yielding a 320-base-pair fragment containing C-region sequences only. This fragment was then subcloned into a PMLC12 plasmid and single-stranded DNA was prepared as described above. For hybridization, the probes were labeled with ^{32}P by primer extension using a primer complementary to the supF portion of the vector downstream of the insert.

RESULTS

Phenotype of Infiltrating Lymphocytes. To obtain activated IL-2-responsive T lymphocytes from sites of inflammation, fragments of synovial tissue were cultured in medium containing recombinant IL-2 (10 units/ml). After expansion in culture, 2×10^7 cells were recovered for analysis of the TCR genomic rearrangements. Initial analyses were performed after 10–28 days in culture and were repeated several times over a period of 6 months. In 13 of 14 cases, the cultured lymphocytes were CD3$^+$ (mean, 91%; range, 86–98%); in case 13 (rheumatoid arthritis), 58% were CD3$^+$. Similarly, 13 of 14 lymphocyte samples were CD4$^+$ (mean, 88%; range 62–98%); in case 6 (osteoarthritis), only 8% were CD4$^+$. The majority of the lymphocytes cultured from each sample were HLADR$^+$ (mean, 91%; range, 77.8–98.8%). These findings substantiate previous observations that T lymphocytes in rheumatoid synovium are predominantly CD4$^+$ and HLADR$^+$ (7, 8). In our study, 11 of 14 samples were from patients with classical rheumatoid arthritis; three (samples 6, 7, and 12) were from patients with a clinical diagnosis of probable osteoarthritis; cells propagated from two of these (samples 7 and 12) were T lymphocytes with surface markers similar to those in the samples derived from patients with classical rheumatoid arthritis. Histologically, sample 12 showed an inflammatory infiltrate consistent with rheumatoid arthritis, whereas samples 6 and 7 showed minimal lymphocytic infiltration and only mild lining cell hyperplasia. Despite the absence of marked lymphocytic infiltration in these samples, it was possible to expand the T lymphocytes in culture with IL-2. HLA typing demonstrated that the patient population was heterogeneous with respect to class I (data not shown) and class II MHC antigens. No DR4$^+$ predominance was noted (only 4 of 11 with rheumatoid arthritis were DR4$^+$), although 9 of the 11 were MC1$^+$ (28).

Southern Blot Analysis of Synovial-Derived Lymphocytes. Southern blot analysis of the DNA from the expanded populations of T lymphocytes in each individual culture showed rearranged TCR β-chain bands. *Eco*RI digests (Fig. 1 *a–c*) hybridized with a C_β probe derived from the Jurβ1 cDNA clone (29) (generously provided to us by Tak W. Mak) revealed no 11-kilobase (kb) germ-line band and the appearance of 1–3 rearranged bands in 13 of the 14 cases. *Eco*RI digests of DNA obtained from sample 6 contained only the 4.2-kb band (Fig. 1*a*). *Eco*RI digests of T-cell DNA derived from two different joints in the same patient (samples 14 a and b) showed identical rearrangement patterns (Fig. 1*c*). Since the use of only one restriction enzyme may be insufficient to detect clonality, we digested each sample of DNA not only with *Eco*RI, but also with *Hin*dIII. Since there is a *Hin*dIII site just 3′ to $J_{\beta1}$ (24, 30), rearrangements detected with this enzyme are likely to have occurred to $C_{\beta2}$. DNA from a polyclonal T-cell population digested with *Hin*dIII shows a germ-line pattern whether a C_β or an entire β-probe is used (20). With the entire Jurβ1 probe, *Hin*dIII digests revealed a germ-line pattern consisting of 3.5-, 8.0- and 13-kb bands corresponding to $C_{\beta1}$, $C_{\beta2}$, and V regions, respectively. Six representative *Hin*dIII-digested samples are

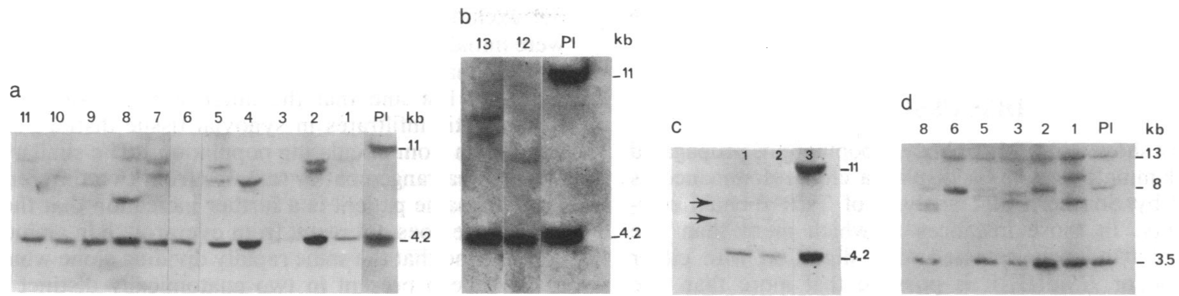

FIG. 1. (*a* and *b*) Southern blot analysis of TCR β-chain rearrangement in uncloned T cells grown and expanded in IL-2-containing medium from synovial fragments from patients with destructive joint disease. Data from 11 of the 14 patients are shown. Each lane number corresponds to the number assigned to each patient. Samples 6 and 7 were from patients with a clinical diagnosis of osteoarthritis. The remainder were from patients with classical rheumatoid arthritis. The DNA from these lymphocytes and another from normal human placenta (Pl), which served as a germ-line control, were digested with *Eco*RI and hybridized to the C_β region probe (*Bgl* II/*Stu* I fragment of the Jurβ1 probe). One to three rearranged bands are evident in each population of T cells except in sample 6, where only the 4.2-kb band is observed. DNA size was determined with a *Bst* II-digested λ phage. Two blots are shown because the samples were run on different gels with slightly different resolution. (*c*) Southern blot analysis of TCR rearrangement in T cells grown from the synovium of two different joints from the same patient with rheumatoid arthritis. *Eco*RI-digested DNA was hybridized to the C_β probe. Lanes: 1, DNA from lymphocytes propagated from the synovium of a metacarpophalangeal joint; 2, DNA from lymphocytes propagated from the synovium of the wrist; 3, human placenta DNA. Both rearranged restriction fragments are shared (arrows). (*d*) Southern blot analysis of TCR β-chain rearrangement in six representative T-cell cultures from synovium from patients with destructive joint disease. The DNA from these lymphocytes was digested with *Hin*dIII and hybridized to the entire Jurβ1 probe. The number of each lane corresponds to the number assigned to each respective patient as in *a*. Pl, human placenta DNA. Evaluation of the germ-line pattern and estimation of the size of the DNA fragments were performed as described in *a*. Samples 1, 3, 5, and 8 show rearranged bands, some in addition to germ-line bands (samples 3, 5, and 8). In sample 6, the faint germ-line 3.5-kb band suggests that the $C_{\beta 1}$ region may have been deleted in the majority of T lymphocytes from this culture.

shown in Fig. 1*c*. In seven samples, the germ-line pattern was preserved, indicating that the rearrangements observed in *Eco*RI digests of the DNA from these cells were to $C_{\beta 1}$. In several other cultures, one or two non-germ-line C-region bands were observed, indicating rearrangements to $C_{\beta 2}$. In the only culture in which $T8^+$ lymphocytes predominated (sample 6), the 3.5-kb band was very faint, which raises the possibility of a deletion of the $C_{\beta 1}$ region on both alleles in the majority of the cultured cells. In all 14 cases, the Jurβ1 V region was observed on the 13-kb germ-line band. Southern blot analysis of DNA in fibroblasts cultured from the rheumatoid synovial samples showed no TCR germ-line polymorphism after digestion with either *Eco*RI or *Hin*dIII (data not shown). Southern blot analysis of both *Eco*RI and *Hin*dIII digests of DNA from all the lymphocyte cultures repeated several times over a period of several months revealed a consistent pattern of TCR β-chain rearrangements. In the three cases in which DNA samples from the lymphocytes were analyzed before and after stimulation with the lectin PHA, the observed band pattern was unchanged.

Southern Blot Analysis of DNA from Blood-Derived Lymphocytes. To exclude the possibility that oligoclonality was the result of an artifact of *in vitro* culture, we established long-term cultures of peripheral blood lymphocytes to determine whether a dominant clone detectable by Southern blot analysis would emerge with time. Peripheral blood lymphocytes obtained from nine healthy individuals were cultured in either medium alone, medium and PHA, medium and recombinant IL-2, medium and unrelated irradiated lymphocytes (which would provide alloantigens in a MLR), or medium and tetanus toxoid (a soluble protein antigen). In the control experiments, T lymphoblasts were separated on Percoll gradients at the time of maximal proliferation to obtain an enrichment in responder cells. To further maintain IL-2 responsiveness, cultures were restimulated at 14-day intervals either with the specific antigen and fresh irradiated autologous mononuclear cells or with unrelated irradiated mononuclear cells and PHA. A total of 102 cultures were established and maintained for at least 42 days; 15 cultures were propagated for 90 days. In all of these cultures, Southern blot analysis of *Eco*RI digests with the C_β probe showed a deletion of the 11-kb band, conservation of the 4.2-kb band, and no rearranged bands. When analysis was performed using the entire Jurβ1 probe, the additional germ-line V-region bands were evident (Fig. 2). *Hin*dIII digests showed only germ-line bands for both C and V regions (data not shown). Together these results suggest that the control cultures were composed of polyclonal T-cell populations and that despite repeated antigen or mitogen stimulation *in vitro*

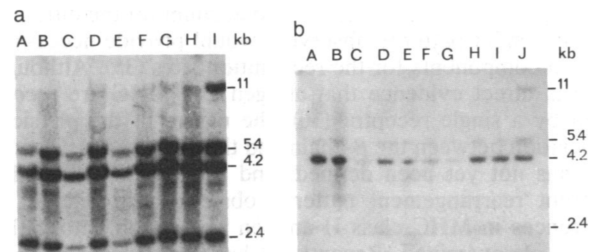

FIG. 2. (*a*) Southern blot analysis of TCR rearrangement in peripheral blood T lymphocytes obtained from healthy donors and propagated in culture for 14 days as described. Each sample of DNA was digested with *Eco*RI and hybridized to the entire Jurβ1 probe. All samples show a depletion of the 11-kb band and conservation of the 4.2-kb band. There is no evidence of a single dominant non-germ-line band. The 5.4-kb and 2.4-kb bands correspond to V regions. Lanes: A, Percoll-separated tetanus toxoid-induced lymphoblasts; B, Percoll-separated MLR-induced lymphoblasts; C, Percoll-separated PHA-induced lymphoblasts; D, tetanus toxoid-induced lymphoblasts; E, MLR-induced lymphoblasts; F, PHA-induced lymphoblasts; G, T lymphocytes propagated in IL-2-containing medium only; H, T cells in culture medium only; I, human placenta DNA. (*b*) Southern blot analysis of TCR rearrangement in peripheral blood T lymphocytes obtained after 42 days in culture and propagated as described. Each sample of DNA was digested with *Eco*RI and hybridized to the entire Jurβ1 probe. In all cases, there was a depletion of the 11-kb band and no detectable non-germ-line band. Lanes: A–C, tetanus toxoid-induced lymphoblasts: A, PHA/unrelated APC restimulation; B, tetanus toxoid/autologous APC restimulation; C, no restimulation; D–F, MLR-induced lymphoblasts; D, PHA/unrelated APC restimulation; E, restimulation with initial alloantigenic cells; F, no restimulation; G–I, PHA-induced lymphoblasts; G, restimulated with PHA and unrelated APCs; H, restimulated with PHA and autologous APCs; I, no restimulation; J, human placenta DNA.

for 90 days, the emergence of a dominant clone could not be detected.

DISCUSSION

Our study shows that T-lymphocyte populations propagated from inflammatory synovia display a clonal dominance as assessed by Southern blot analysis of TCR β-chain rearrangements. In those instances in which more than one rearranged band was detected after digestion with either *Eco*RI and/or *Hin*dIII, it is possible that more than one dominant clone was present. Alternatively, two rearranged bands observed after either restriction enzyme digestion could represent a productive and a nonproductive rearrangement in a single clone (31, 32). It is unlikely that two productive rearrangements took place in a single clone, since there is evidence that at the protein level only one allele is expressed in any given cell (33–35). Similarly, it is unlikely that all of the observed bands represent only nonproductive D–J rearrangements, since common nonproductive rearrangements detectable by Southern blot analysis are not reported in heterogeneous T-cell populations and have not been observed in our control studies. Furthermore, preliminary results from studies using pools of V-region probes show that a V-region probe may hybridize to at least one of the rearranged fragments observed using C-region probes, thus indicating that some of the rearranged bands correspond to a productive rearrangement (data not shown). The differences in the rearrangement patterns observed among different individuals are not surprising since T cells may use different V_B gene segments for the recognition of a given antigen or, alternatively, T lymphocytes with different antigen specificities may utilize the same V_β gene segment (34). If there is a common antigen responsible for T lymphocyte proliferation in rheumatoid arthritis, it is possible that sequences generated by combinatorial joining of different V_β and J_β gene segments, which would account for the different rearrangement patterns observed, would provide necessary β-chain components for the recognition sites (35). Although there is direct evidence that antigen and MHC are recognized by a single receptor (36), the nature of the physical association between the β-chain and the antigen–MHC complex has not yet been defined, and it is possible that the different rearrangement patterns observed are related to differences in MHC class II antigen expression among the individuals examined. Recently, it has been suggested that the β-chain may form the most important contact points with MHC ligands (37). It is noteworthy that in the instance in which two separate synovial samples were obtained simultaneously from two different joints, *Eco*RI-digested DNA showed shared rearranged bands (Fig. 1*d*), while *Hin*dIII digests retained the germ-line pattern (data not shown). Furthermore, when another synovial sample was divided into five fragments and activated T cells were grown out of each fragment separately, the TCR β-chain rearrangements observed were the same in all five cultures (data not shown), suggesting that the same dominant clone observed was present in different areas of the synovial membrane. Preliminary evidence from analysis of other inflammatory infiltrates indicate that oligoclonality is not likely to be limited to joint disease but may represent an important feature of inflammatory as well as malignant lymphocytic infiltrations.

There are several interpretations of our findings that the infiltrating lymphocytes in the synovium from patients with destructive joint diseases are oligoclonal. Such oligoclonality might be due to an *in vitro* artifact resulting from the more rapid outgrowth of a limited number of cells when expanded in culture. We showed, however, in control cultures of peripheral blood lymphocytes, that even after 90 days, oligoclonality was not detected. Although these results do not exclude outgrowth of a few rapidly dividing cells that were initially present in the inflamed synovial tissue, they do indicate that the frequency of such cells in the blood must be relatively low and that the outgrowth of clones from the lymphocytic infiltrates in synovial tissue distinguishes this population from circulating populations. The similar pattern of TCR rearrangement in samples from two different joints from the same patient is a further indication that the clonal dominance does not result from overgrowth in an individual culture, and that the same rapidly dividing clone would have to have been present in two anatomically distinct cellular infiltrates. It is unlikely that our results reflect merely a paucity of activated cells that could respond to IL-2 at the start of culture. While the intensity of inflammation estimated from histological analysis of representative sections varied among samples cultured, in order to obtain 2×10^7 T cells after only 10 days in culture, assuming a doubling time of 24 hr (the maximum rate of proliferation that could be sustained for a week in culture), it would be necessary to have initiated the cultures with at least 1×10^4 activated T cells.

A second possibility is that during the process of inflammation in the joint there is a selective activation of a "dominant" lymphocyte population. The chronic inflammation might result in a strong selective process over a period of many months, during which specificity and susceptibility to immune regulation would exert highly selective forces on the inflammatory infiltrate. Such selection could conceivably be characteristic of a wide variety of inflammatory stimuli, not restricted to rheumatoid arthritis, and it could explain our findings in the patients with clinical osteoarthritis. Recently, it has been shown that the immune response to 2,4,6-trinitrophenol in a mouse H-2k^d-restricted system is dominated by the generation of clones with identical β and α chains (38). This observation suggests that selective processes act on the responding T-cell population, resulting in the skewing of the TCR for antigen–MHC and in the dominant expression of one TCR. An analogous situation may arise in inflammatory responses to antigens in human disease, whereby in response to a given antigen–MHC complex, T cells with certain TCRs have a proliferative advantage over other T-cell clones with the same specificity. The rearrangement of TCR germ-line segments may not be random, and the selection of self-MHC restriction and tolerance may favor T cells expressing certain receptors (37). During the immune response, stimulation with antigen may preferentially expand a population of T cells expressing certain TCRs. It is noteworthy that antigen-stimulated T-cell populations *in vitro* remained polyclonal, suggesting that although the antigenic epitopes may be limited, the responding T-cell repertoire is diverse; selective pressures *in vivo* would thus be necessary for dominant clones to emerge.

A third possibility is that the observed oligoclonality reflects the presence of a limited number of potentially responding lymphocytes as a fundamental feature of autoimmune diseases such as rheumatoid arthritis. Since the process of autoaggression may result from the proliferation of a limited number of lymphocytes that escape the normal state of tolerance to self-tissue antigens, the oligoclonality detected by the DNA analyses may reflect the limited number of TCR rearrangements that correlate with specificities associated with the breakage of tolerance. Even if the autoaggression results from the response to a foreign antigen, as in rheumatic heart disease, the immune component cross-reactive to self may show limited diversity.

Our study shows that T-cell clonality is not restricted to lymphoproliferative diseases but may be a feature of certain inflammatory processes as well. The ultimate proof of the *in vivo* significance of the dominant clones will require their identification *in situ* and an assessment of their specificity

and function in modulating the joint disease. Still, regulation of the number and function of these lymphocyte clones could have a significant impact on the local immune response and may represent an important avenue for future modulation of local immunity.

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