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Research Article

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Differential Expression of Ia Antigens by Rheumatoid Synovial Lining Cells

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Abstract

The differential expression of Ia antigens was studied in freshly isolated rheumatoid nonlymphoid synovial lining cells (SLC) and rheumatoid synovial fibroblast cell lines cultured in the presence of Interferon-γ, using a large panel of anti-Ia reagents with monomorphic or polymorphic specificities. All the HLA-DR or -DQ specificities detectable on the corresponding peripheral blood B cells were also expressed in freshly isolated SLC. However, in all instances, the number of DR-positive SLC exceeded the percentage of cells expressing DQ antigens. In addition, the epitope expression of Ia antigens varied within the DR or DQ populations of Ia molecules as revealed by polymorphic reagents. Double-label experiments or using the ingestion of Latex particles as a marker demonstrated that the synovial macrophages (type I SLC) primarily bear the DR^+DQ^+ phenotype, while there is an additional population of nonphagocytic SLC (previously termed type II SLC) that has a DR^+ and monocyte marker negative phenotype but did not have detectable levels of DQ antigens as analyzed by both fluorescence microscopy and cell sorter analysis. This latter population frequently had a morphology showing dendritic processes and rapidly lost the expression of Ia antigens upon culture. Cells with a similar, primarily DR^+ phenotype were readily obtained in synovial fibroblast cultures after treatment with Interferon-γ. These data suggest that there are two populations of Ia^+ synovial lining cells: the synovial macrophages (type I cells) with the DR^+DQ^+ phenotype, and cells probably related to fibroblasts with a DR^+ phenotype without detectable DQ antigens (type II cells). The fact that the latter phenotype could be induced by Interferon-γ treatment of cultured synovial fibroblasts suggests that this mediator may have a similar role in vivo in the activation of certain synovial cell populations.

Introduction

One of the hallmarks of histological findings in the rheumatoid synovium is the marked hyperplasia of the synovial lining, resulting in a multicellular layer of synovial cells and the invasion of cartilage and bone structures by cells presumably derived from these populations. With immunological reagents to probe the structure of the rheumatoid synovial membrane, the most striking finding proved to be the intense expression of Ia antigens (class II HLA molecules) on the majority of synovial lining cells (1–4). A further dissection of this heterogenous cell population demonstrated that the majority of Ia-positive cells belong to the monocyte/macrophage lineage. These cells, termed "type I" and identifiable with the type A synovial cells, bear typical monocyte lineage antigens, express Fc receptors, and frequently have a morphology characterized by an elongated cell shape with long cell processes (1, 5). This cell population is found both in the rheumatoid synovium and within apparently normal synovial tissue derived from patients with noninflammatory joint diseases (1, 2).

An additional Ia-positive cell population, detectable primarily in the rheumatoid synovium and termed "type II" cells, is characterized by the intense expression of these class II molecules, but also by the absence of markers characteristic of the monocyte lineage. These cells frequently have a dendritic shape and lose the expression of Ia antigens upon culture (1, 5). The origin of these cells is not clear. Whereas the Ia-positive phenotype along with their dendritic morphology would make them similar to the classic dendritic cells described by Steinman et al. (6), the facts that the majority of synovial dendritic cells are poor inducers of the mixed leukocyte reaction (7), rapidly lose Ia antigens upon culture (1, 5), and produce large amounts of collagenase (8, 9) would rather suggest a relationship to the fibroblast lineage.

A possible aid to delineate the origin of these cells may be the analysis of Ia antigen expression, in particular studying the gene products of the various Ia loci. This is suggested by the findings that not all Ia molecules are expressed in equal amounts on the cell surface. Thus, a divergent expression of DR and DQ class II molecules has been described on peripheral blood monocytes (10) and certain tissue cells (11). In addition, investigating the differential Ia antigen expression of synovial cells after incubation with Interferon-γ, which has recently been shown to be a potent Ia antigen inducer on these cells (12), may shed light on a possible role of this mediator in an in vivo situation.

Methods

Patient population. 12 patients (nine female, three male) with definite or classic rheumatoid arthritis according to American Rheumatism Association criteria were studied. The majority of patients had received antiinflammatory nonsteroidal drugs that had been discontinued at least 12 h before surgery. Patients on a current treatment with gold salts, other remission-inducing drugs, or steroids were excluded from study.

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Two synovial specimens from amputation material from patients without apparent joint diseases were included as normal counterparts to the patient group with rheumatoid arthritis. Peripheral blood monocytes were derived from nine patients with rheumatoid arthritis and nine normal donors.

Processing of synovial tissue and cell isolation. Synovial tissue obtained at reconstructive surgery was finely minced and incubated with RPMI medium 1640 containing 1 mg/ml collagenase (Sigma Chemical Co., St. Louis, MO) and 0.15 mg/ml DNAse (Sigma Chemical Co.), as previously described in detail (1). After 60–90 min at 37°C, the resulting cell suspension was purified upon Ficoll/Isopaque density gradients (1). Nonlymphoid synovial cells (SLC)[1] were enriched by means of a discontinuous Percoll (Pharmacia Inc., Piscataway, NJ) density gradient, as reported previously (1). Cells were harvested from the interface between 20 and 50% Percoll, washed three times, and stored in RPMI 1640 supplemented with 10% fetal calf serum (FCS) or cultured in tissue culture flasks, respectively, to study the cell morphology. This cell population contained < 5% of cells with lymphoid characteristics as determined by morphology or staining with monoclonal antibodies directed towards T or B lymphocytes (see below). The viability exceeded 95%. Peripheral blood non–T cells were separated using neuraminidase-treated sheep red blood cells (E) (13).

Peripheral blood monocytes with a purity of > 90% as determined by reagent Leu M3 (14) were obtained using Nycodenz (Nyegaard Diagnostica, Oslo, Norway) gradient (*d* 1.068 g/ml) centrifugation of peripheral blood mononuclear cells previously incubated in autologous EDTA-plasma according to product literature.

Peripheral blood B cells were isolated from monocyte-depleted peripheral blood mononuclear cells using Nylon wool columns resulting in a cell population with > 80% surface immunoglobulin-positive cells (13).

Cell cultures and identification of cell populations. Synovial lining cells were cultured in two ways. (*a*) Immediately after isolation, SLC were distributed to 24-well tissue culture plates (Nunc, Roskilde, Denmark) containing medium RPMI 1640, supplemented with 10% FCS, and allowed to adhere to glass cover slips (Bellco Glass, Inc., Vineland, NJ), which had previously been placed into the wells, for ~ 3 h (5). Latex particles were added to identify phagocytic cells (1). Subsequently, the cover slips were taken out, washed thoroughly, and used for immunofluorescence staining as previously described (15). Immediately after the final washing step, the cover slips were examined by means of a Epifluorescence Microscope (Orthoplan II; E. Leitz, Inc., Rockleigh, NJ) equipped with phase-contrast optics.

(*b*) SLC were cultured in regular culture flasks for about two or three passages until the proportion of cells bearing monocyte markers or Ia antigens was < 1% as determined by the antimonocyte reagent Leu M3 (=Mϕ P-9) or by OKIa 1. Subsequently, cells were split into equal portions with one part cultured as before and the second part treated with 200 U/ml of recombinant Interferon-γ (kindly provided by Dr. Papendieck, Thomae Co., Biberach, FRG, and produced by Genentech Inc., San Francisco, CA) for a period of 7 d. Preliminary kinetic experiments with concentrations of 1–1,000 U/ml and periods of 1–9 d had indicated that maximum Ia antigen expression was achieved using these culture conditions (data not shown). After culture, cells were trypsinized, stained in parallel, and Ia antigen expression was studied using both the fluorescence microscope and the fluorescence-activated cell sorter.

Identification of cell surface antigens. Characteristics of the monoclonal anti-Ia reagents are outlined in Table I. This panel of antibodies included reagents that react with common framework antigens of the DR antigen family (reagents HLA-DR = L 243; OKIa 1), the DQ antigen family (Leu 10), or an epitope shared by both (22 c 6) in addition to reagents that detect epitopes restricted to polymorphic Ia determinants belonging to either the DR (IL-R2, 109 d6, 16.23) or the DQ (Genox 3.53, IV D12) antigen family (for references and sources see Table I).

Cells belonging to the monocyte/macrophage lineage were detected by reagent Leu M3 (=Mϕ P-9 [14]) either in indirect immunofluorescence or using this reagent tagged with phycoerythrin (Becton Dickinson & Co., Röderbach, FRG) in direct staining. T cells were identified by staining with the monoclonal reagent 89 b 1 (pan–T cell antibody [26]), whereas B cells were detected by surface immunoglobulin staining (27). All the reagents described above were used in concentrations that had been shown in extensive preliminary studies to be saturating using the fluorescence-activated cell sorter.

Immunofluorescence. The aforementioned monoclonal reagents were used in indirect immunofluorescence as previously reported, either utilizing the fluorescence microscope (E. Leitz, Inc.; 200 Watt light bulb, epiillumination system with optimal optical alignment) or the EPICS V fluorescence-activated cell sorter (Coulter Electronics Inc., Hialeah, FL) (1). In all experiments, isotype-specific control monoclonal antibodies with irrelevant specificities (directed towards viral antigens or the acetylcholine receptor) were included. In all instances, the determination of positive cells was performed by both microscopy and using the fluorescence-activated cell sorter. The microscopic method allows the precise determination of the presence or absence of surface immunofluorescence from whole cellular fluorescence by the technique of varying the plane of focus. Experiments showing an extensive background staining in the cell sorter, a common problem when applying synovial cells to this instrument, were excluded from evaluation.

The standard run conditions with fluorescein isothiocyanate–stained cells using the cell sorter were as follows throughout all experiments. The laser intensity was 250 mW on the wave length of 488 nm. Cells were identified by the small angle lightscatter (FALS) with the light intensity reduced by a 10% neutral density filter; the gain setting on the FALS amplifier was 2. The FITC-fluorescent light was gated on this signal. To separate these frequencies from the excitation light, a 515-nm interference filter combined with a 515-nm absorbance filter was used. In front of the photomultiplier (PMT), a 560-nm short pass filter was inserted to induce Raman scattering. The PMT setting was 700, equalling 1,400 V. In each run, 10,000 cells were measured. Therefore, the two spectra were comparable with regard to cell number. To calculate the percentage of positive cells, we divided the sum of the positive events in each channel (=Pi) by the total cell number according to the following formula:

$$\% \text{ positive fluorescence} = \left(\sum_{i=1}^{256} P_i / \sum_{i=1}^{256} T_i\right) \cdot 100,$$

where channel = i; test spectrum = T; T_i = number of events in channel i in test spectum; control spectrum = C; C_i = number of events in channel i in conrol spectrum. $P_i = T_i - C_i$, if $T_i - C_i > 0$. Otherwise, $P_i = 0$. To measure fluorescence intensity, we used the following standard formula:

$$F = (\sum P_i \cdot i / \sum P_i) = \text{median channel.}$$

Thus, the median channel was calculated after subtraction of the control events. This mode of evaluation, originally outlined in the "Immuno Program" (Coulter Electronics Inc.), was chosen because of the considerable background staining of synovial cells and proved to result in identical percentages of positive cells when compared with conventional counting by fluorescence microscopy.

In the result section, cells that were recorded as negative by cell sorter analysis and fluorescence microscopy with regard to staining with the anti-DQ reagents will subsequently be referred to as DQ^-. To compare fluorescence intensities, a logarithmic scale is contained within the cell sorter profiles (*x*-axis). For example, fluorescence intensity of cells recorded at scale point 100 is five times higher than that of cells at scale point 20.

1. *Abbreviations used in this paper:* FALS, small angle light scatter; FITC, fluorescein isothiocyanate; MC, median channel; PMT, photomultiplier; SLC, nonlymphoid synovial lining cell.

Table I. Characterization of Anti-Ia Monoclonal Antibodies Used

Antibody	Specificity	DR associations	Isotype	Reference	Source
Monomorphic reagents					
OKIa 1	DR		IgG 2	16	Ortho
HLA-DR (L243)	DR		IgG $2a_k$	17	ATCC
22c6	DR, DQ		IgG 2a	13	Own
Leu 10	DQw1, w3	1, 2, 4, 5, 6, 8, 9, w10	IgG 1_k	18	Becton-Dickinson & Co.
Polymorphic reagents					
IL-R2	DRw52 (MT2)	3, 5, w6,8	IgG $2b_k$	19	Coulter Electronics, Inc.
109d6*	DRw53 (MT3)	4, 7, w9	IgG 2a	20	Own
16.23‡	DR3 (w6)		IgG 3	21	Personal gift
Genox 3.53	DQw1 (MB1)	1, 2, (6, 8)	IgG 1	22	ATCC
IVD 12	DQw3 (MB3)	4, 5, (w6, w9)	IgG 1	23	Personal gift

* Reagent 109d6 detects an epitope on a DR beta-chain present on all DRw53 cells, but also on some DR1 cells (20, 24). ‡ Reagent 16.23 detects an epitope on a DR beta-chain primarily present on DR3 cells, but also on some DRw6 cells (21, 25). Ortho, Ortho Pharmaceutical, Raritan, NJ. ATCC, American Type Culture Collection, Bethesda, MD.

Results

Expression of Ia antigen epitopes by freshly isolated synovial lining cells. Fig. 1 shows the percentages of freshly isolated SLC positive for the various anti-Ia reagents obtained in eight representative patients in whom a complete evaluation was performed. In all experiments, the proportion of DR^+ SLC as determined by both monomorphic and polymorphic reagents exceeded the number of DQ^+ cells. Fig. 2 illustrating results from two representative patients demonstrates that not only the number of DR^+ cells was higher than that of the DQ^+ cells, but also the staining intensity of the DR^+ cells was higher as documented by cell sorter analysis.

In all instances, the typing for the polymorphic Ia specific-

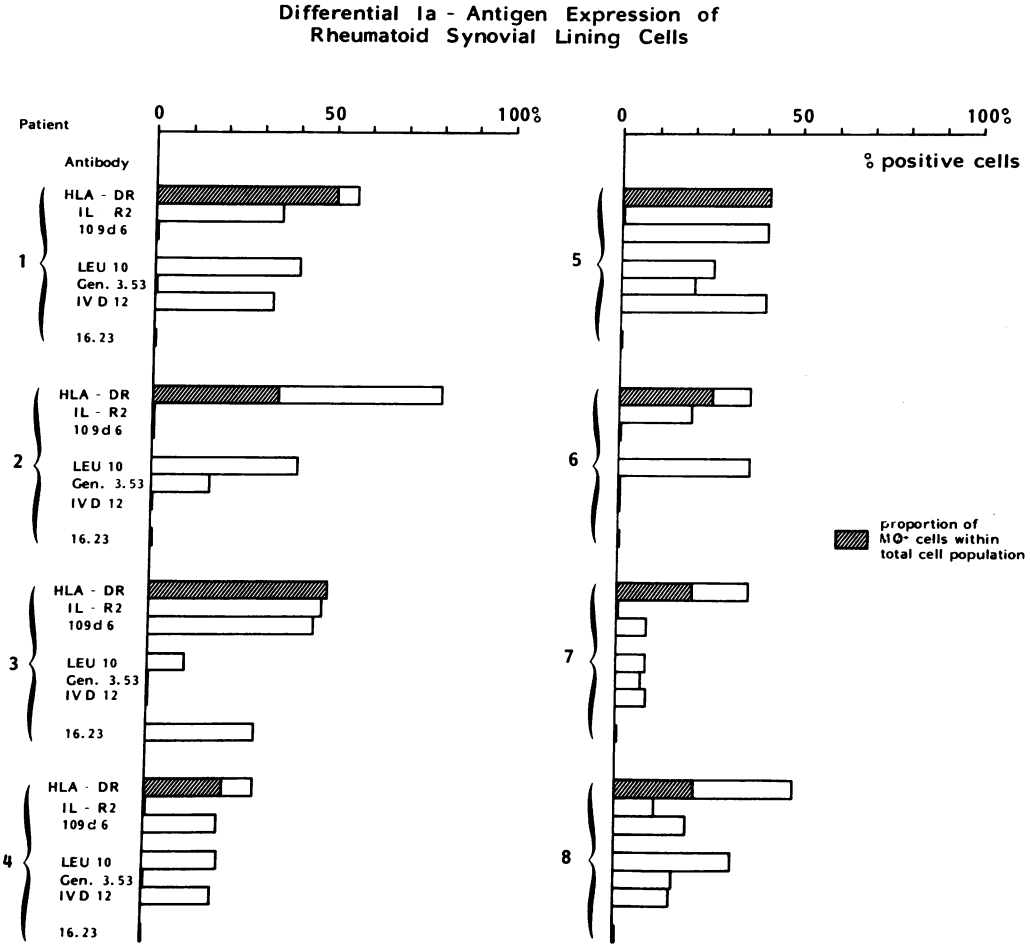

Figure 1. Percentages of Ia-positive nonlymphoid synovial lining cells from eight representative patients with rheumatoid arthritis as determined by various monoclonal reagents directed towards public or private Ia antigen determinants. (*Hatched bars*) Proportion of cells bearing monocyte lineage antigens within the total cell population. These cells were subsequently shown to be HLA-DR^+ (Fig. 5) and, therefore, their proportion was integrated into the HLA-DR^+ bar.

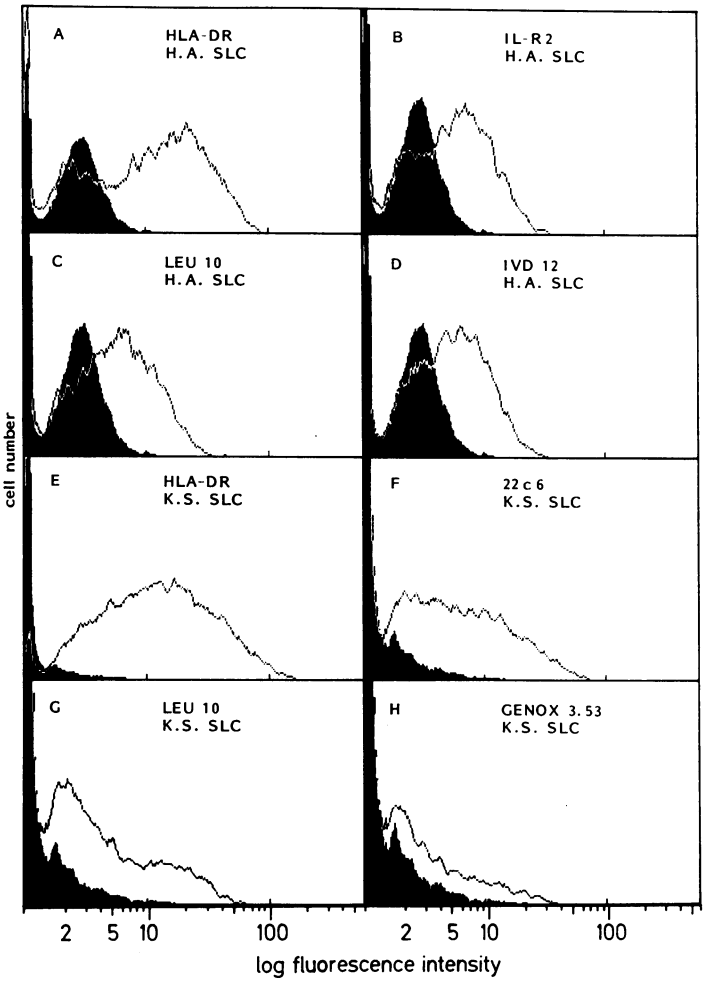

Figure 2. Cell sorter profiles describing the distribution of Ia^+ synovial lining cells (SLC) from two patients with rheumatoid arthritis (patients H.A. and K.S.). (*Solid areas*) Background staining obtained with monoclonal reagents of irrelevant specificity. (*Dashed curves*) Ia^+ cells. In both experiments, the number and staining intensity of DQ^+ cells was less than the percentage of DR^+ cells. Patient H.A.: reagent HLA-DR, 56% positive cells with a median channel (MC) of 114; Leu 10 39%/MC 87; IL-R2 35%/MC 87; IVD12 34%/MC 84. Patient K.S.: HLA-DR 81%/MC 101; Leu 10 42%/MC 71; 22c6 52%/MC 79; Genox 3.53 17%/MC 66.

ities on SLC was in accordance with results determining positivity on the corresponding peripheral blood non–T cells (data not shown). However, of special interest, the number of positive cells as determined by the monomorphic reagents HLA-DR or Leu 10, respectively, was frequently higher than the percentages of positive cells as measured by the polymorphic reagents identifying epitopes belonging to the same Ia antigen family. Thus, for instance in patient 1 in Fig. 1, 58% of the cells expressed the epitope detected by reagent HLA-DR, whereas only 35% of cells were positive for antibody IL-R2 detecting the DRw52 specificity (see Table I). Because of the individual HLA type of this patient, the cells were negative for reagent 109 d6.

Similar data within the DQ antigen family were obtained in patient 2 (Leu 10^+ cells > Genox 353^+ cells) and patient 8 (Leu 10^+ cells > Genox 353^+ cells, IV $D12^+$ cells). These data cannot simply be explained by different antibody affinity, because in some instances (e.g., patient 5) identical or even higher percentages of positive cells were obtained with the polymorphic reagents. Fig. 1 also demonstrates that the proportion of SLC bearing the monocyte/macrophage marker Leu M3 (=Mϕ^+) was smaller than the number of DR^+ SLC but, in most experiments, similar to the percentage of DQ^+ cells. Taken together, these data indicate the presence of a monocyte/macrophage marker negative, but DR^+ SLC population in patients with rheumatoid arthritis.

In two normal synovial tissues derived from amputation material, the number of DR^+ cells was highly similar to the percentage of DQ^+ cells (36% HLA-DR^+/39% Leu 10^+, and 40%/40%, respectively) and nearly identical to the number of Leu $M3^+$ cells (40% in both patients).

Parallel experiments investigating the Ia antigen expression of peripheral blood monocytes revealed that in normal donors (n = 9) 85±16% of the cells were DR^+ (reagent HLA-DR) and 27±24% expressed DQ antigens (reagent Leu 10), whereas in patients with rheumatoid arthritis (n = 9) 74±13% of the cells were DR^+ and 20±28% DQ^+. Fig. 3 *A* shows the cell sorter profile of differential Ia antigen expression in peripheral blood monocytes from a normal donor, demonstrating the lower percentage as well as the lesser staining intensity of the DQ^+ cells when compared with synovial cells.

In contrast to blood monocytes, peripheral blood B cells exhibited similar staining patterns for DR and DQ antigens (Fig. 3 *B*) indicating that the lesser fluorescence intensity in blood monocytes is not due simply to lower antibody affinity.

Synovial cells with macrophage characteristics primarily bear the DR^+DQ^+ phenotype; Ia^+ cells without these markers are DR^+DQ^-. The observation of a divergence of DQ^+ and DR^+ cells, as described above, led to the investigation of the Ia phenotype expression of the monocyte marker positive and negative synovial lining cell populations. In one set of experiments using SLC cultured for 3 h on cover slips, cells with

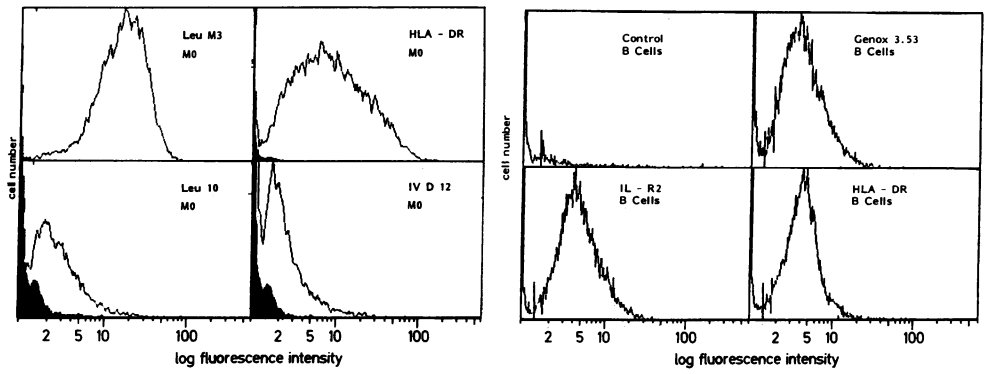

Figure 3. (*A*) Cell sorter profiles of peripheral blood monocytes (*Mo*) derived from a normal donor and separated by Nycodenz. 95% of cells are Leu M3$^+$. 92% of cells are positive for HLA-DR with a MC of 85. The number of DQ$^+$ cells is markedly lower as determined by reagents Leu 10 (30%/MC 48) or IV D 12 (37%/MC 42). Note the lesser staining intensity especially of reagents Leu 10 and IV D 12 when compared to synovial cells (Fig. 2) as indicated by the lower median channels. (*B*) Cell sorter profiles of a peripheral blood mononuclear cell population highly enriched in B cells (85% surface immunoglobulin positive) derived from a patient with rheumatoid arthritis. Note the similar percentages and staining intensities of DQ$^+$ cells (Genox 3.53, 75%/MC 61) and DR$^+$ cells (IL-R2, 81%/MC 63; HLA-DR, 75%/MC 60). Upper left panel shows staining with an irrelevant control monoclonal antibody.

macrophage characteristics were identified by the ingestion of Latex particles, and subsequently, cover slips were stained with the reagents HLA-DR and Leu 10, respectively. Fig. 4 illustrates that both phagocytic and nonphagocytic SLC are HLA-DR$^+$ (Fig. 4, *A* and *B*), whereas only the phagocytic cells depicted in Fig. 4, *C* and *D* were positive for antibody Leu 10. Frequently, the nonphagocytic HLA-DR$^+$ cells had numerous cell processes identifiable by changing the focus of the phase-

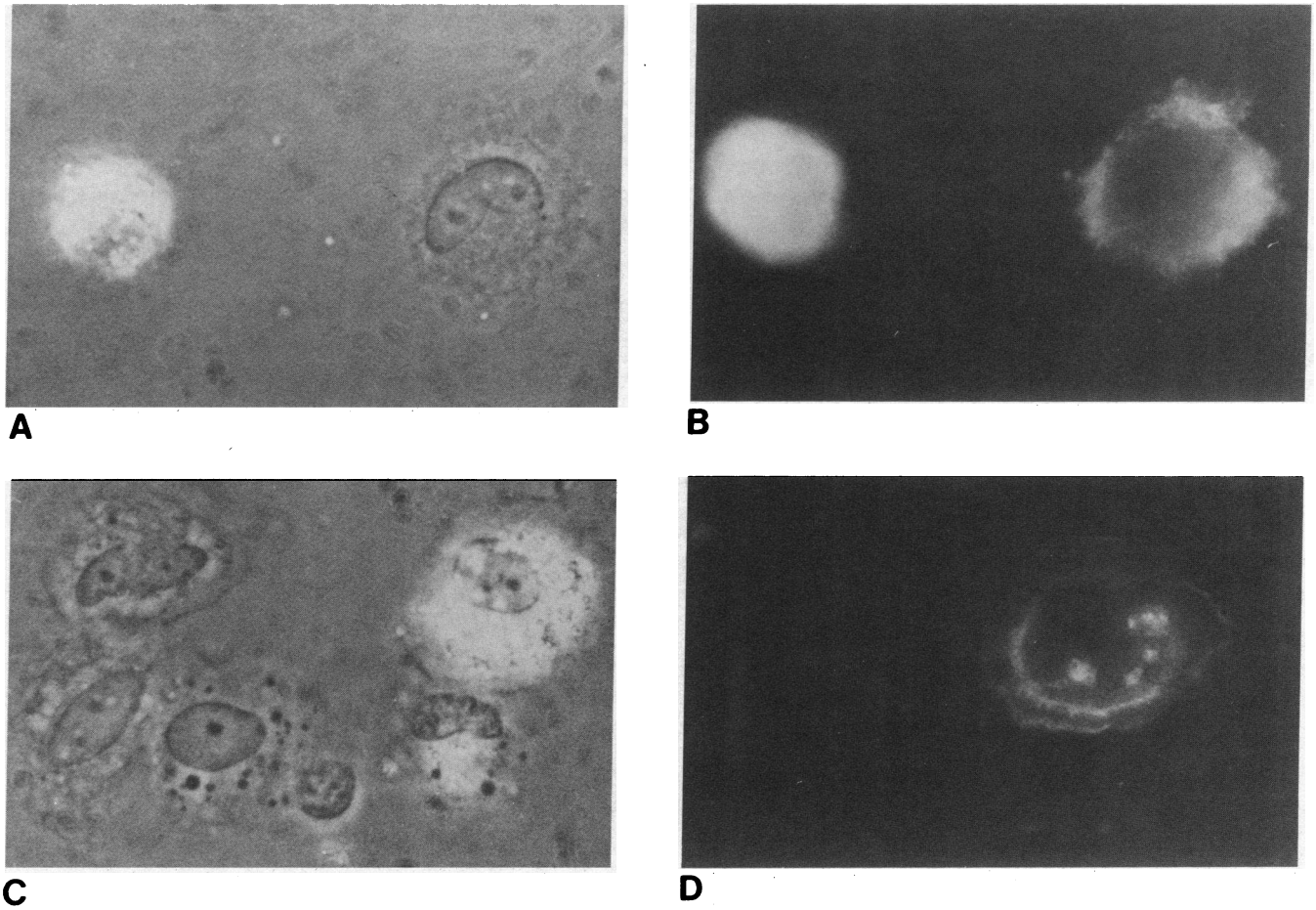

Figure 4. Phase-contrast micrographs and the corresponding immunofluorescence staining of rheumatoid synovial cells adherent to cover slips after ingestion of Latex particles. Staining with antibody HLA-DR reveals that both the nonphagocytic and the phagocytic cells express DR antigens (*A* and *B*). The nonphagocytic cell in *A* is binucleate and has numerous cell processes visible after changing the plane of the phase-contrast microscope, indicating a stellate or dendritic appearance. Using reagent Leu 10, only the phagocytic cells were positive (*C* and *D*). The second phagocytic cell (*C* and *D*) stained weakly for Leu 10 and was not recorded on the film.

Table II. Nonphagocytic Rheumatoid Synovial Lining Cells Are Primarily of HLA-DR$^+$ Leu 10$^-$ Phenotype

Patient	Anti-Ia Reagent	Latex$^+$Ia$^+$	Latex$^+$Ia$^-$	Latex$^-$Ia$^+$	Latex$^-$Ia$^-$
1	HLA-DR	23*	7	27	43
	Leu 10	26	4	8	62
2	HLA-DR	36	0	45	19
	Leu 10	22	14	4	60

* Percentage of total cells falling under the column heading that are DR or DQ positive (first and third data columns) or that are DR or DQ negative (second and fourth data columns).

contrast microscope indicating a dendritic morphology. However, because of the rapid loss of Ia antigens on cultured dendritic cells (1, 5), a short culture period of 3 h, which did not allow the cells to develop the full dendritic appearance, was chosen. Table II summarizes data obtained in two representative patients. They demonstrate that the nonphagocytic Ia$^+$ cells primarily express the DR$^+$DQ$^-$ phenotype, while the majority of phagocytic cells expresses both DR and DQ antigens.

In a further experiment, double labeling was performed using the fluorescence-activated cell sorter. SLC were first stained with reagents HLA-DR or Leu 10, respectively, and then developed by fluorescein rabbit anti-mouse antibodies. Subsequently, the Leu M3 antibody tagged with phycoerythrin was added. The results of this double marker analysis are outlined in Fig. 5 and Table III. They demonstrate that the vast majority of the Mϕ^+ cells bear both DR and DQ antigens, whereas most of the Mϕ^-Ia$^+$ cells are DQ$^-$. Of the Mϕ^+ cells, 91% were DR$^+$ and 89% DQ$^+$, while of the Mϕ^-Ia$^+$(DR$^+$) cells only 30% also expressed the DQ$^+$ phenotype.

Induction of Ia antigens on cultured synovial fibroblasts primarily leads to a DR$^+$DQ$^-$ phenotype. Because Interferon-γ has been shown to be a mediator that induces Ia antigens on synovial cells outside of the monocyte lineage (12), the differential Ia antigen expression was studied on synovial fibroblasts cultured in the presence of this mediator. Before incubation with Interferon-γ, these synovial cells had been both Ia and monocyte marker negative after several passages in culture, during which the initially present Ia$^+$ and Mϕ^+ cells had disappeared. Table IV demonstrates that while DR antigens including the polymorphic specificities were readily inducible on 41–83% of synovial fibroblasts after culture with Interferon-γ, only a small percentage of cells or even no cells expressed DQ antigens. An interesting exception was patient F.S., described in experiment 2. 65% of cells were DQ$^+$, as detected by reagent Leu 10 (Table IV and Fig. 6). The DQ epitopes determined by antibodies Genox 353 and IV D12, both known to be on separate Ia β-chains (22, 23), were also detectable on this patient's cells. This fact makes an "aberrant" Leu 10 epitope expression in a different Ia antigen setting very unlikely.

Figs. 6 and 7 illustrate the direct comparison of Ia antigen expression between freshly isolated synovial lining cells and the synovial fibroblast lines treated with Interferon-γ derived from the same patients. They demonstrate that there was a different Ia antigen distribution in both populations. While in the freshly isolated cells there was a broad range of cells from very weak to extremely bright staining, there was a more peak-like staining pattern of cultured Ia$^+$ synovial fibroblasts after incubation with Interferon-γ. Of particular interest are the divergent patterns of the Leu 10 staining in the two patients described. In patient B.P. (Fig. 7) there were only a few dimly stained Leu 10$^+$ synovial fibroblasts in comparison to brightly positive fresh SLC. In contrast, in patient F.S. (Fig. 6) both populations intensely expressed the Leu 10 epitope.

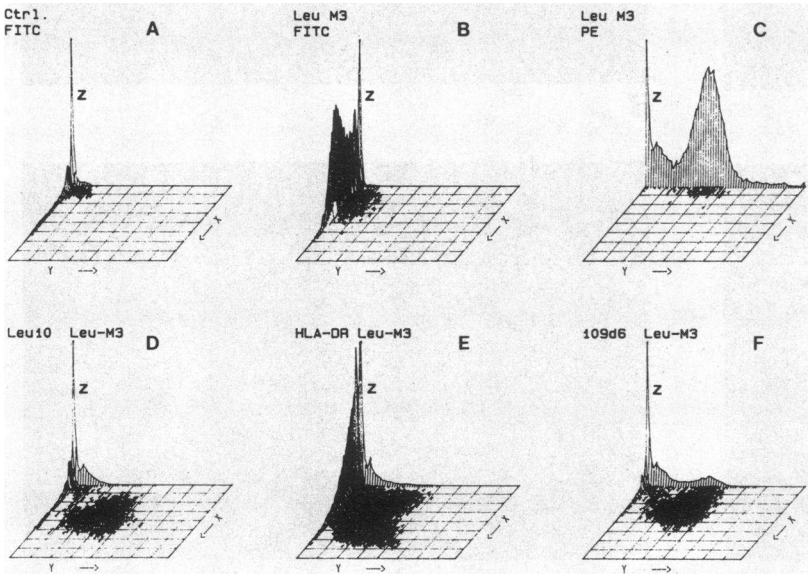

Figure 5. Double-marker analysis of synovial lining cells from a patient with rheumatoid arthritis using combinations of anti-Ia reagents and the anti-monocyte antibody Leu M3. In the panels, *x*-axis shows the intensity of the fluorescein-labeled cells, and *y*-axis denotes the intensity of the phycoerythrin-labeled cells, whereas the *z*-axis indicates the number of positive cells. Cells within the rectangular area stain positively both for fluorescein and phycoerythrin. (*A*, *B*, and *C*) Single marker analysis. (*A*) Staining with an irrelevant monoclonal antibody developed by a fluorescein-labeled serum. (*B*) Staining with Leu M3 alone (fluorescein, *x*-axis). (*C*) Staining with Leu M3 alone directly tagged with phycoerythrin (*y*-axis; red fluorescence; red negative control profile similar to negative shoulder curve in [*E*]). Note that in these panels only very few cells are recorded in the opposite axes, indicating the specificity of the instrument setting for distinguishing the different wavelengths emitted. Both analyses using Leu M3 either developed by fluorescein or phycoerythrin show a similar distribution of positive cells demonstrating the reproducibility of both systems. (*D*, *E*, and *F*) Double-marker analysis. (*D*) Combined staining with Leu 10 (fluorescein, *x*-axis) and Leu M3 (phycoerythrin, *y*-axis), demonstrating that the population of Leu 10$^+$ cells and Leu M3$^+$ cells is nearly identical. (*E*) Double staining with reagents HLA-DR (*x*-axis) and Leu M3 (*y*-axis), demonstrating that nearly all Leu M3$^+$ cells are positive for antibody HLA-DR but that there is an additional population (recorded on the *x*-axis) of HLA-DR$^+$ Leu M3$^-$ cells. (*F*) Combined staining with reagents 109d6 (*x*-axis) and Leu M3 (*y*-axis) reveals the presence of a 109d6$^-$, but Leu M3$^+$ population. The percentages of these double-marker analyses are shown in Table III.

Table III. Double-Label Experiments Demonstrating That Mϕ⁻/Ia⁺ Cells Primarily Bear HLA-DR⁺ Leu 10⁻ Phenotype

	Mϕ⁺Ia⁺	Mϕ⁺Ia⁻	Mϕ⁻Ia⁺	Mϕ⁻Ia⁻
DR	42*	4	20	34
Leu 10	41	5	6	48

Mϕ detected by reagent Leu M 3 (MϕP-9). * Percentage of total cells falling under the column heading that are DR or DQ positive (first and third data columns) or that are DR or DQ negative (second and fourth data columns).

Discussion

The rheumatoid synovial tissue offers a unique opportunity to study activation mechanisms involved in an intense in vivo immune reaction that is characterized by the presence of large numbers of activated macrophages (1, 5), activated T and B lymphocytes (13, 28–30) and other cell populations (8, 9). The marked expression of Ia antigens on many of these cells allows the investigation of a possible role of these gene products in immunoregulatory processes in an inflammatory reaction and may serve as a base line to study Ia induction mechanisms on the gene level. Three principal findings emerged from the present study.

(*a*) There are two types of Ia⁺ synovial lining cell populations that can be discriminated on the basis of the level of DQ antigen expression: first, type I synovial cells with all characteristics of macrophages that express both DR and DQ antigens, and second, the monocyte marker negative type II cells characterized by the presence of large amounts of DR antigens, but low or undetectable amounts of DQ antigens.

(*b*) Interferon-γ primarily induces the expression of DR antigens on synovial fibroblasts, while there are only small amounts of DQ antigens in the majority of experiments.

(*c*) These results are further confirmed using reagents with polymorphic anti-Ia specificities; they demonstrate a heterogeneity of Ia epitope expression within the DR or DQ Ia antigen families. In addition, staining with antibodies IL-R2 and 109 d6 show the presence of the second DR β-chain on both freshly isolated SLC and on synovial fibroblasts after treatment with Interferon-γ.

In the present investigation, extensive efforts were made to allow a reproducible evaluation of positive cells and their degree of fluorescence intensity. Due to their large size and surface volume as well as their autofluorescence caused by cytochromes contained within the cytoplasm, synovial cells give a rather high background. This problem is best overcome using the fluorescence microscope, where it is relatively easy to distinguish between the typical rim-like staining of positive cells and the dull nonspecific whole cellular fluorescence or the cytochromes that are located in large spots within the cytoplasm frequently lighting up in different colors. Fluorescence microscopy, however, does not allow a precise quantitation of fluorescence intensity, which is best done by flow cytometry. Therefore, using fluorescence-activated cell sorter analysis, a computer program normalized the test spectrum to a control spectrum obtained with irrelevant control monoclonal antibodies of the same isotypes. With this combined approach, the data obtained by flow cytometry and conventional microscopy were always in accordance. To allow for a direct comparison of positive cells using the various monoclonal antibodies, all evaluations of flow cytometry were kept constant using the same gain throughout the experiments. Therefore it is possible that synovial cells with a very low DQ antigen density may have been missed in the cell sorter due to the fluorescence background or in the microscope where the low antigen density may have escaped visual evaluation. Despite these technical limitations, the type I and type II synovial cell populations are still very distinct, with the first population having large, easily recordable amounts of DQ antigens and the second one with possibly very low but not detectable levels of DQ antigens. The similar antigen densities of DR and DQ antigens on peripheral blood cells (Fig. 3 *B*) strongly argue against simply a lower antibody affinity of all three reagents used to detect DQ antigens (Leu 10, Genox 3.53, and IVD12).

In contrast to synovial macrophages characterized in the present report, peripheral blood monocytes—while DR⁺ in the majority of cells—express only small amounts of DQ antigens. This has been shown in several previous studies (10, 11, 31) and was confirmed also in the rheumatoid situation in the present investigation. While entering the synovial tissue, blood monocytes apparently receive activation and/or differentiation signals that lead to an enhanced DR antigen expression as well as to a newly acquired expression of DQ antigens. This is not only true in an inflammatory reaction, but also within apparently normal synovial tissue as shown in the present report. Several observations suggest that these activation/differentiation mechanisms are not uniform in the various tissues. Thus, alveolar macrophages, while intensely expressing both DR and DQ antigens, display a markedly reduced expression

Table IV. Predominant Expression of DR-Antigens on Rheumatoid Synovial Fibroblasts after Incubation with Interferon-γ

	DR Antigens				DQ Antigens		
Experiment	HLA-DR	IL-R2	109d6	16.23	Leu 10	Genox 353	IVD12
1	83*	0	68	0	10	0	6
2	75	0	54	0	65	33	18
3	70	0	20	0	11	0	19
4	58	12	18	52	11	0	12
5	52	0	0	0	0	0	0
6	41	ND	ND	ND	0	ND	ND

* Percentage of positive cells.

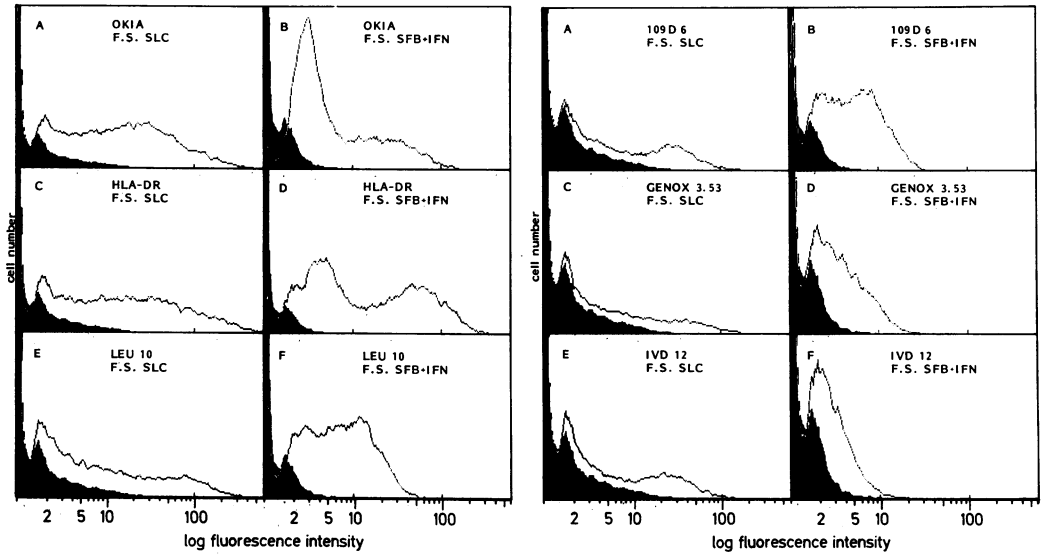

Figure 6. (*A*) Direct comparison of Ia antigen analysis of SLC and synovial fibroblast cell lines (SFB) in patient F.S. using monomorphic reagents. In contrast to patient B.P. (Fig. 7), there is a considerable number of Leu 10^+ cells in both populations (panels *E* and *F*; SLC 32% positive/MC 97, SFB 65%/MC 76). (*B*) These results are confirmed using reagents with polymorphic specificities towards DQ antigens (panels *C–F*).

of certain monocyte lineage antigens, including Leu M3 (31). On the other hand, liver Kupffer cells do not bear DQ antigens, but stain brightly for DR antigens (11). A possible candidate for an activation signal in the rheumatoid synovium appears to be Interferon-γ, which has been shown to induce DQ antigens on peripheral blood monocytes in addition to an enhanced DR antigen expression (10, 11). The presence of Interferon-γ has been documented both in the rheumatoid synovium (32) and the synovial fluid (33). A possible source of this mediator may be the large number of activated Ia^+ T cells present in this compartment (13, 27, 34), especially in view of the finding that Ia^+ T cells are the primary producers of Interferon-γ in in vitro assays (35).

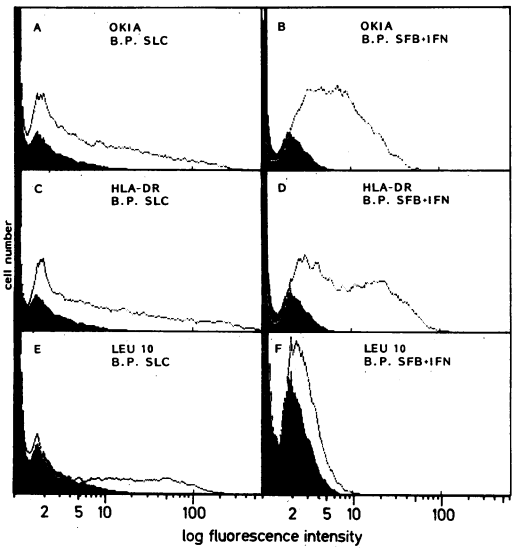

Figure 7. Direct comparison of anti-Ia staining of freshly isolated synovial cells (*SLC*) and the same patient's corresponding synovial fibroblast line (*SFB*) incubated with Interferon-γ. Note the different distribution of Leu 10^+ cells (*E* and *F*) of fresh synovial cells stained intensely (MC 134) and the dimly stained (MC 42) synovial fibroblasts, while in both preparations there was a large number of $OKIa^+$ and $HLA\text{-}DR^+$ cells.

The second Ia^+ synovial cell population has a completely different phenotype. It does not bear typical monocyte/macrophage markers or detectable levels of DQ antigens while intensely expressing DR antigens. Many of these cells have a dendritic morphology. An interesting parallel to these cells is found in synovial fibroblast cultures that have been treated with Interferon-γ. These cells have a similar Ia phenotype in as much as they show a low expression of DQ antigens. These findings suggest that the majority of the freshly isolated Ia^+/monocyte marker negative type II synovial cells belong to the fibroblast lineage. In this respect, data presented by Klareskog et al. (36) showing the presence of DR^+DQ^- cells in pannus tissue from patients with rheumatoid arthritis are of special interest. Again, as in the case of synovial macrophages, Interferon-γ could be a possible mediator inducing Ia antigens on synovial fibroblasts that are normally Ia negative. A similar aberrant Ia antigen expression has been demonstrated on chondrocytes in certain joint diseases, including rheumatoid arthritis (37, 38), on thyrocytes in autoimmune thyroiditis (39), on keratinocytes in lichen ruber planus (40), and on other cell types in various autoimmune diseases (41, 42). This expression of class II HLA antigens has been suggested to trigger the development of autoimmune diseases (43) and could be of particular importance in rheumatoid arthritis characterized by the accumulation of intensely Ia^+ cells in the target tissue. Of special interest, Interferon-γ does not have a uniform effect on the induction of Ia antigens. Thus, in contrast to cells belonging to the monocyte or B cell lineage, this mediator does not induce DQ antigens at all on cells of epithelial lineage (11) and varying but usually low amounts of these surface molecules on cells of mesenchymal lineage such as chondrocytes (44) or fibroblasts as shown in the present report.

It will be of particular interest to study the molecular basis of the differential Ia antigen induction by Interferon-γ on cells of mesenchymal lineage. Thus, as shown in the present report, most synovial fibroblasts express only low amounts of DQ antigens after Interferon-γ treatment, while in some instances these antigens were intensely expressed (Fig. 7). These data are very similar to results obtained with human articular chondrocytes incubated with Interferon-γ (44) and to observations in skin fibroblast and endothelial cell cultures with IFN-γ,

where only some cultures became DQ^+, expressing only 10–20% of the amount of DR antigens (45). The significance of this discoordinate Ia antigen expression is not clear, but data demonstrating different roles of Ia antigen families as restriction elements in helper cell and suppressor cell function (46–50) suggest a particular role of DR or DQ antigens also in the autoimmune situation.

Besides altering the HLA phenotype, Interferon-γ has been shown to decrease the synthesis of types I and III collagens and fibronectin in adherent synovial cells (12). In chondrocytes, Interferon-γ treatment changes the expression of certain surface molecules (44). Therefore, the action of this important T cell–derived lymphokine may induce an intricate change of synovial cells resulting in both an induction of certain immune responses and changes of the synovial microenvironment leading to the tissue destruction characteristic of rheumatoid arthritis.

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