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NOTES

Beta Interferon Is Produced by *Chlamydia trachomatis*-Infected Fibroblast-Like Synoviocytes and Inhibits Gamma Interferon-Induced HLA-DR Expression

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Infection of fibroblast-like synovial cells with *Chlamydia trachomatis* (serotype D strain IC Cal 8) in culture induced the secretion of beta interferon (IFN-β). Chlamydial infection inhibited IFN-γ-induced expression of HLA-DR antigen in the cells. Addition of IFN-β antibody directly to infected cultures mitigated HLA-DR inhibition, suggesting involvement of produced IFN-β.

Chlamydia trachomatis, an obligate intracellular parasite, is a frequent cause of sexually transmitted diseases and a known triggering agent of reactive arthritis. Although chlamydiae cannot be cultivated from the joint chlamydial antigens, DNA and RNA have been detected in synovial tissue of reactive arthritis patients (11, 14, 16). In recent studies, chlamydial RNA and atypical forms of chlamydiae were identified by in situ hybridization and gold labeling immunoelectron microscopy within fibroblasts and macrophages in subintimal layers of the synovial membrane (5, 20). These findings support the hypothesis of inapparent chlamydial infection in reactive arthritis that may be associated with the persistence of *C. trachomatis* in a noncultivable state within synovial cells (SC).

C. trachomatis has a biphasic growth cycle. Infectious elementary bodies (EBs) enter the host cell and differentiate into larger reticulate bodies (RBs). These RBs divide by binary fission within the expanding endosome, resulting in development of an intracellular chlamydial inclusion. After a period of growth, RBs reorganize into new infectious EBs that are released by host cell lysis or exocytosis.

It has been suggested that persistent chlamydial infections are associated with reversible alterations of the chlamydial growth cycle (3). Interferons in particular have been implicated in restriction of facultative and obligate intracellular bacteria. *Chlamydia*-specific T lymphocytes of the Th1 subset which produce gamma interferon (IFN-γ) were identified in the synovial fluid of patients with reactive arthritis (12, 25). In several permanent cell lines and in epithelial cell cultures, IFN-γ treatment arrests chlamydiae at the EB stage or induces atypical RBs that do not differentiate into new EBs (9). In addition, IFN-α and IFN-β were found to inhibit intracellular chlamydial growth (21). IFN-γ is an inducer of major histocompatibility complex class II (MHC II) molecules on several cell types that are not conventional antigen-presenting cells. IFN-β can inhibit IFN-γ-induced MHC II expression and may function as a modulator of localized immune responses in inflammation (6). In healthy joints, synovial fibroblasts do not express MHC II molecules, but synoviocytes of patients with rheumatoid arthritis show an abundant MHC II expression and are able to act as antigen-presenting cells (7, 8). The synovial fibroblast possibly represents a cell type in which chlamydiae can persist in reactive arthritis. In nonprofessional phagocytes, the chlamydial inclusion does not fuse with endosomes and lysosomes. Lysosomal markers are absent within the chlamydial vacuole (13). This may result in a failure of antigen processing and presentation by MHC II molecules. Moreover, a chlamydial infection may modulate the IFN-γ-induced MHC II expression in these cells.

In this study, we investigated whether fibroblast-like SC produce IFN-β in response to *C. trachomatis* infection in culture and whether IFN-β can inhibit chlamydial growth in these cells. Furthermore, we evaluated the influence of *C. trachomatis* infection on IFN-γ-induced expression of HLA-DR molecules in fibroblast-like SC.

Human SC cultures were established from synovial biopsies obtained during meniscusectomies and arthroscopies of traumatic joint disease patients as previously described (22). Briefly, the tissue specimens were dissected into small pieces and digested in Iscove modified Dulbecco medium (IMDM; Biochrom, Berlin, Germany) containing 2 mg of collagenase type II (Biochrom) per ml. The cells were grown in IMDM supplemented with 30% fetal calf serum (FCS; Biochrom) together with 100 U of penicillin per ml and 100 μg of streptomycin (Sigma, Deisenhofen, Germany) per ml. Synoviocytes that were used during passages 4 to 12 were characterized as fibroblast-like cells by staining with monoclonal antibody to prolylhydroxylase (clone 5B5; Dako, Hamburg, Germany [22]).

High-titer stocks of *C. trachomatis* serotype D strain IC Cal 8 (obtained from the Institute of Ophthalmology, London, United Kingdom) were propagated in McCoy cell monolayers in serum-free medium SF-3 (Cytogen, Berlin, Germany) containing 1 μg of cycloheximide per ml (4). Infected cells were collected in phosphate-buffered saline (PBS) with 0.2 M sucrose and 2% FCS 48 h after infection and lysed by sonication. The suspension was centrifuged at 800 × *g* for 10 min to re-

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TABLE 1. IFN-β release from fibroblast-like SC infected with various doses of *C. trachomatis* serotype D[a]

Subject no.[b]	IFN-β (U/ml)[c]			
	Mock infected	MOI		
		5	10	50
1	—[d]	11 (2)	18 (5)	28 (2)
2	—[d]	14 (5)	26 (7)	41 (8)

[a] Supernatants were collected at 48 h after infection.
[b] Experiments were performed with cell cultures established from different patients.
[c] The data represent the means (standard deviations) of two wells.
[d] —, not detectable.

TABLE 2. Time course of IFN-β production by SC infected with *C. trachomatis* serotype D[a]

Subject no.[b]	IFN-β (U/ml)[c] at h after infection:			
	24	48[d]	72	96
1	9 (1)	28 (6)	26 (7)	16 (4)
2	8 (1)	24 (8)	20 (4)	11 (2)
3	26 (4)	37 (5)	44 (11)	18 (4)

[a] Cells were infected at an MOI of 20.
[b] Experiments were performed with cell cultures established from different patients.
[c] The data represent the means (standard deviations) of two wells.
[d] The mean values at 48 h were compared to the mean values of 24 h (paired *t* test, $P \leq 0.03$). The mean paired difference was calculated from the paired differences of the mean values of subjects 1, 2, and 3.

move cell debris. Supernatants were stored at −70°C. Infectivity titers were quantified by titrating the number of inclusion-forming units (IFU) per ml in McCoy cells. These titers were used to determine the multiplicity of infection (MOI) for SC.

SC were grown in 11-mm-diameter culture tubes containing a glass coverslip (Sarstedt, Nürnbrecht, Germany). Cultures were checked for *Mycoplasma* contamination by DNA staining (bisbenzimidazole; Biochrom). Chlamydial stocks were diluted in PBS, and an inoculum of 0.2 ml was added to the culture tubes. SC monolayers (8×10^4 to 10×10^4 cells/tube) were infected by centrifugation at $4{,}000 \times g$ at 37°C for 1 h at different MOIs (IFU per cell). After the inoculum was decanted, the cells were washed in medium to remove nonadsorbed chlamydiae and further incubated with 0.5 ml of IMDM containing 10% FCS but no antibiotics. For mock-infected cultures, synoviocytes were centrifuged with a harvest of uninfected McCoy cells. Culture supernatants of infected and mock-infected cells were collected, centrifuged at $14{,}000 \times g$ for 5 min, and stored at −70°C.

Interferon was determined by a microtiter method based on the inhibition of the cytopathic effect of vesicular stomatitis virus (VSV) on human WISH cells (1). Briefly, serial dilutions of samples and IFN-β standard (Biochrom) were incubated on human WISH cells (ATCC CCL 25) for 20 h at 37°C and then challenged with VSV. Virus inhibition was measured by a colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay 24 h later (19). To identify IFN-β in the bioassay, culture supernatants were preincubated with 100 nU of polyclonal sheep antibody to human IFN-β (Chemicon, Harrow, United Kingdom) per ml at room temperature for 1 h.

The effect of IFN-β on the production of infectious chlamydiae was tested in a growth yield assay. Confluent SC monolayers were incubated in IMDM supplemented with 10% FCS and IFN-β (50 to 200 U/ml). After 24 h, the cells were infected at an MOI of 1. After 72 h, infected monolayers were scraped from the coverslips into 0.5 ml of saccharose phosphate buffer (PBS with 0.2 M saccharose and 2% FCS) and briefly sonicated to release chlamydiae. Monolayers of McCoy cells were infected with serial dilutions of the disrupted SC suspensions and incubated in serum-free SF-3 medium containing 1 μg of cycloheximide per ml for 48 h. Chlamydial inclusions were visualized by immunofluorescence staining with fluorescein isothiocyanate (FITC)-conjugated antibody to *C. trachomatis* major outer membrane protein (MOMP) (Syva Microtrak *C. trachomatis* culture confirmation test; Behring Diagnostics, Inc., Marburg, Germany) to determine the titer of IFU per milliliter.

To induce expression of HLA-DR molecules, mock-infected SC and cells infected at an MOI of 5 were incubated in IMDM with 10% FCS and 10 U of IFN-γ per ml for 48 h. To determine an influence of IFN-β on IFN-γ-induced HLA-DR expression, infected cells were treated with 10 U of IFN-γ per ml and 100 nU of polyclonal sheep antibody to human IFN-β per ml. In control tubes, mock-infected cells were treated with 50 U of IFN-β per ml and 10 U of IFN-γ per ml for 48 h.

HLA-DR and *Chlamydia* antigens were detected by double immunofluorescence staining. Cells on coverslips were fixed in acetone for 30 min and air dried. The cells were incubated with monoclonal mouse antibody to human HLA-DR (clone DK 22; Dako) at a dilution of 1:50 in PBS containing 1% bovine serum albumin (BSA) at room temperature for 60 min. The coverslips were washed in PBS and incubated with biotinylated rabbit anti-mouse immunoglobulin G Fab_2 fragment (Dako) at a dilution of 1:400 in PBS with 1% BSA for 1 h. After being washed with PBS, the cells were incubated with RPE-conjugated streptavidin (Dako) diluted 1:20 in PBS with 1% BSA for 30 min. To visualize chlamydial inclusions, the cells were incubated with FITC-conjugated antibody to *C. trachomatis* MOMP and Evans blue. After 30 min, the cells were washed again, mounted in PBS, and examined under a fluorescence microscope with excitation at 490 nm. The percentage of cells expressing HLA-DR molecules was determined by examining about 200 cells per coverslip.

For flow cytometric analysis, the cells were detached by use of EDTA (0.2 mM), washed twice in PBS, and incubated with phycoerythrin-conjugated monoclonal antibody to HLA-DR (Becton Dickinson, Hamburg, Germany) at room temperature for 20 min. Two additional washes were performed, and la-

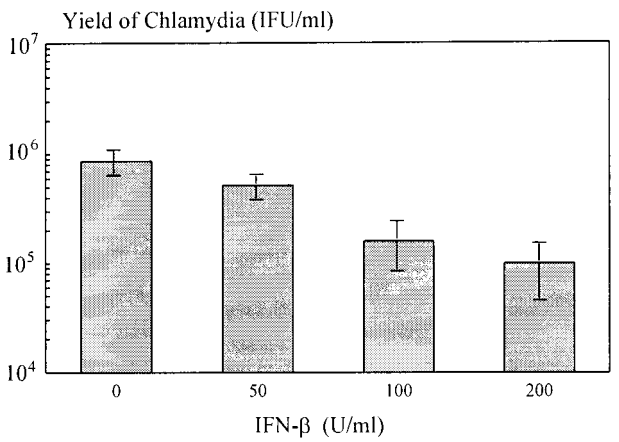

FIG. 1. Effect of IFN-β on chlamydial yield in SC. Values are the means and standard deviations of three different experiments.

TABLE 3. HLA-DR expression in fibroblast-like SC stimulated with IFN-γ and infected with *C. trachomatis* serotype D

Infection type	Treatment	HLA-DR-positive cells (%)[a]
Mock	Medium	—[b]
Mock	10 U of IFN-γ per ml	91 (5)
Mock	10 U of IFN-γ per ml plus 50 U of IFN-β per ml	61 (7)[c]
C. trachomatis (MOI of 5)	10 U of IFN-γ per ml	50 (15)[c]
C. trachomatis (MOI of 5)	10 U of IFN-γ per ml plus 100 nU of anti-IFN-β per ml	79 (4)[d]

[a] Average number (standard deviation) of four separate experiments.
[b] —, not detectable.
[c] Significantly different from values for mock-infected cells after IFN-γ stimulation (Welch *t* test, $P \leq 0.01$).
[d] Significantly different from values for infected cells after IFN-γ stimulation (Welch *t* test, $P \leq 0.01$).

beled cells were analyzed with a FACScan (Becton Dickinson) flow cytometer and CELL QUEST software. A total of 10,000 cells was scored in each sample.

In culture supernatants of *Chlamydia*-infected fibroblast-like synoviocytes, interferon activity was found by the VSV inhibition assay, whereas mock-infected cells did not release biologically active interferon (Tables 1 and 2). Interferon in culture supernatants was characterized as IFN-β, because specific antibody to IFN-β completely neutralized the activities. Maximal levels of IFN-β activity were detected at 48 to 72 h after infection (Table 2).

Infection of fibroblast-like SC with *C. trachomatis* resulted in intracellular growth characterized by the development of inclusion bodies. Infection at an MOI of 1 resulted in about 17% inclusion-positive cells and in production of new infectious chlamydiae. Treating the cells with IFN-β had a slight effect on chlamydial growth. IFN-β (200 U/ml) caused an eightfold reduction of chlamydial yield (Fig. 1).

Synovial fibroblasts did not express HLA-DR in mock-infected and *Chlamydia*-infected cultures. Incubation with a low dose of IFN-γ (10 U/ml) induced expression of HLA-DR in about 90% of the cells (Table 3 and Fig. 2A). When synoviocytes were infected with *C. trachomatis* IC Cal 8 and then incubated with IFN-γ, the percentage of HLA-DR-positive cells was reduced in comparison to that for mock-infected cultures (Table 3 and Fig. 2B). The percentage of *Chlamydia*-mediated inhibition of IFN-γ-induced HLA-DR expression was 45% when the cells were infected at an MOI of 5. The inhibition depended on the infectious dose. At lower MOIs of 1 and 2, the number of HLA-DR-expressing cells did not vary between infected and mock-infected cultures. It is known that IFN-β can counter the stimulatory effect of IFN-γ on expression of MHC II antigen in several cell types (6). Coincubation of synoviocytes with IFN-β (50 U/ml) and IFN-γ (10 U/ml) for 2 days reduced the expression of HLA-DR (Table 3). The percentage of inhibition was 30%. Addition of anti-IFN-β antibody directly to infected cultures mitigated but did not abolish IFN-γ-induced HLA-DR expression (Table 3). The percentage of HLA-DR inhibition was reduced to 10%. In infected cultures, the percentage of HLA-DR-positive cells did not significantly differ between cells with a chlamydial inclusion and cells without an inclusion. Since acetone fixation of cells can destroy surface antigens, unfixed cells were stained with HLA-DR antibody and analyzed by fluorescence-activated cell sorting. In reference to negative controls, a boundary for HLA-DR-positive cells was defined at a fluorescence intensity of 10^3 (Fig. 3A and B). Chlamydial infection at an MOI of 5 or 10

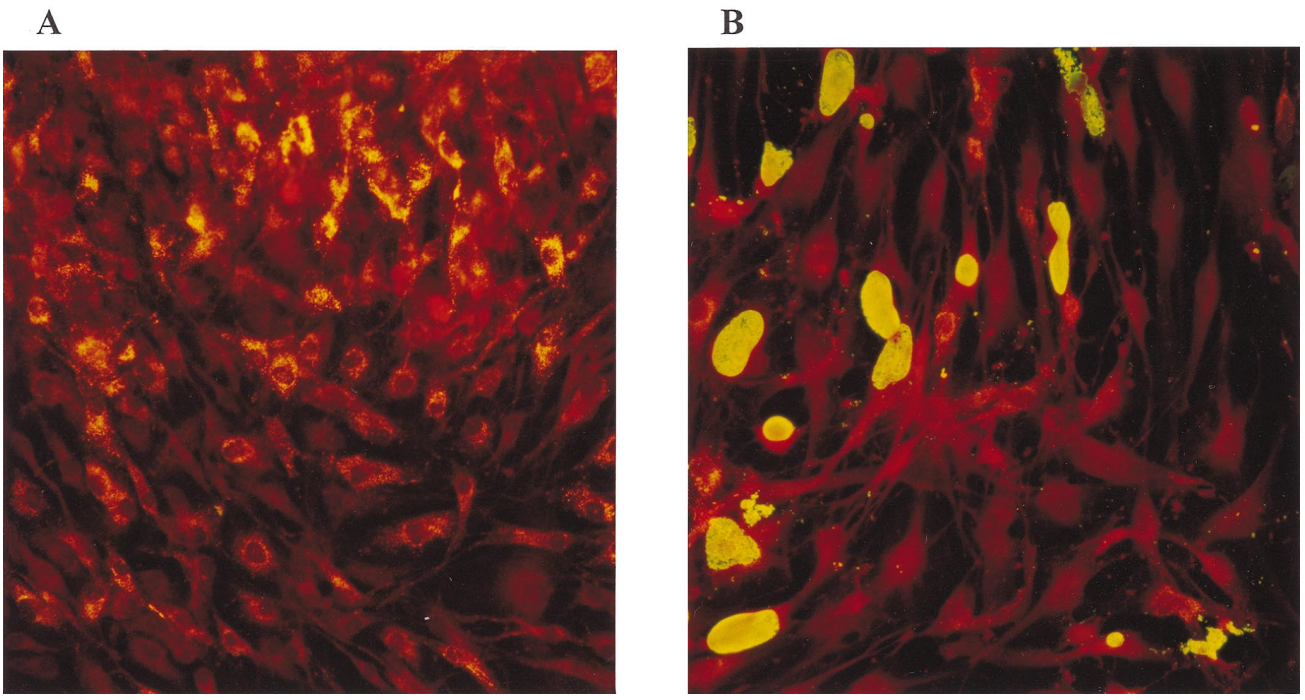

FIG. 2. Expression of HLA-DR molecules in *C. trachomatis*-infected cultures of SC detected by immunofluorescence staining. (A) Incubation with 10 U of IFN-γ per ml for 48 h induces HLA-DR molecules as indicated by the granular staining of SC. Cells were stained for HLA-DR by indirect immunofluorescence with an RPE-conjugated secondary antibody. Magnification, ca. ×200. (B) Following chlamydial infection and incubation with IFN-γ, a smaller number of SC show positive staining for HLA-DR antigen. Chlamydial inclusions were stained with FITC-conjugated antibody to MOMP. Magnification, ca. ×200.

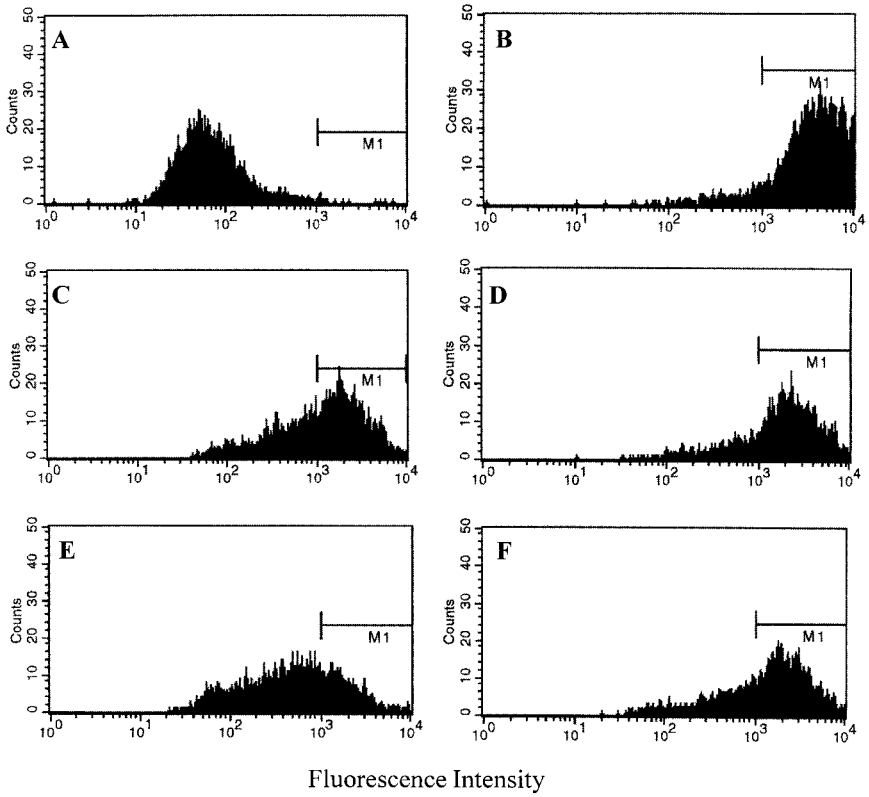

FIG. 3. Reduction of IFN-γ-induced HLA-DR expression on synovial fibroblasts after infection with *C. trachomatis*. (A) Mock-infected cells incubated in culture medium without IFN-γ (0.4% positive cells; total mean fluorescence intensity [MFI] = 97). (B) Mock-infected cells stimulated with 10 U of IFN-γ per ml (92% positive cells; MFI = 4,190). (C) *Chlamydia*-infected cells (MOI of 5) stimulated with 10 U of IFN-γ per ml (59% positive cells; MFI = 1,658). (D) *Chlamydia*-infected cells (MOI of 5) treated with IFN-γ (10 U/ml) and IFN-β antibody (100 nU/ml) (77% positive cells; MFI = 2,366). (E) *Chlamydia*-infected cells (MOI of 10) stimulated with 10 U of IFN-γ per ml (28% positive cells; MFI = 830). (F) *Chlamydia*-infected cells (MOI of 10) treated with IFN-γ (10 U/ml) and IFN-β antibody (100 nU/ml) (68% positive cells; MFI = 2,026). Flow cytometric analysis was performed after 48 h of incubation of cell cultures.

reduced the percentage of DR-expressing cells from 92 to 60 or 28%, respectively (Fig. 3C and E). This effect was mitigated by neutralizing IFN-β activity in the cultures (Fig. 3D and F).

Fibroblast-like SC represent a cell type in which bacteria may persist in reactive arthritis. Investigations of *Yersinia enterocolitica*-induced arthritis have shown that yersiniae can invade and persist in synoviocytes in culture (15). In this work, we have reported that synoviocytes can also be infected with *C. trachomatis*. Infection of fibroblast-like cells resulted in production of IFN-β, which has a slight inhibitory effect on the production of infectious chlamydiae in these cells. The interferon-induced inhibition of chlamydial growth is characterized by the induction of indoleamine 2,3-dioxygenase, which catalyzes the degradation of tryptophan to kynurenine (2). Tryptophan is an essential amino acid, and a depletion of its intracellular pool is responsible for alterations in the growth cycle of *Chlamydia* (2). It has been reported that IFN-γ strongly stimulates indoleamine 2,3-dioxygenase activity in synoviocytes, while IFN-β has a weak stimulatory effect (18). This observation may explain the minor effect of IFN-β on chlamydial growth in synoviocytes. The in vivo mechanisms of chlamydial persistence have not been defined. IFN-γ produced by *Chlamydia*-reactive T lymphocytes might contribute to an inapparent infection of the synovial membrane in reactive arthritis (12, 25).

IFN-γ induced the expression of HLA-DR molecules in fibroblast-like synoviocytes. An IFN-γ concentration of 10 U/ml was sufficient to induce HLA-DR expression in about 90% of the cells and corresponds to levels found in the synovial fluid of patients with chronic arthritis (10). The expression of HLA-DR antigen on synovial fibroblasts in rheumatic diseases was repeatedly described. In rheumatoid arthritis, osteoarthritis, and traumatic damage, HLA-DR is expressed not only on macrophages but also on fibroblasts of the synovial membrane (24). Furthermore, it has been shown that synovial fibroblasts can possess an antigen-presenting capacity. *Mycobacterium tuberculosis*-reactive CD4 T cells that were isolated from synovial fluid of rheumatoid arthritis patients could be stimulated by IFN-γ-treated synovial fibroblasts as antigen-presenting cells (7). Infection of fibroblast-like SC with *C. trachomatis* significantly reduced the IFN-γ-induced expression of HLA-DR molecules. IFN-β was identified as a counterregulatory cytokine in MHC II expression (6). When infected cells were simultaneously incubated with IFN-γ and with a neutralizing antibody to IFN-β, the inhibition of HLA-DR expression was mitigated. We conclude that endogenously induced IFN-β is involved in HLA-DR inhibition. These results are consistent with in vitro studies on human cytomegalovirus-infected endothelial cells (23). The molecular mechanism of this antagonism between IFN-γ and IFN-β has not been fully elucidated. IFN-γ-induced MHC II transcription depends on class II transactivator. IFN-β acts in part by reducing the functional competence of class II transactivator for transactivating MHC II promoters (17).

Besides professional antigen-presenting cells and T lymphocytes, synovial fibroblasts probably play an important role in

the immunopathogenesis of reactive arthritis. When *Chlamydia*-infected cells do not express HLA-DR antigen, the ability of the immune system to detect these cells may be impaired.

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