Presentation of Arthritogenic Peptide to Antigen-Specific T Cells by Fibroblast-Like Synoviocytes

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Objective. To assess the ability of rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) to function as antigen-presenting cells (APCs) for arthritogenic autoantigens found within inflamed joint tissues.

Methods. Human class II major histocompatibility complex (MHC)–typed FLS were used as APCs for murine class II MHC–restricted CD4 T cell hybridomas. Interferon-γ (IFNγ)–treated, antigen-loaded FLS were cocultured with T cell hybridomas specific for immunodominant portions of human cartilage gp-39 (HC gp-39) or human type II collagen (CII). T cell hybridoma activation was measured by enzyme-linked immunosorbent assay of culture supernatants for interleukin-2. Both synthetic peptide and synovial fluid (SF) were used as sources of antigen. APC function in cocultures was inhibited by using blocking antibodies to human class II MHC, CD54, or CD58, or to murine CD4, CD11a, or CD2.

Results. Human FLS could present peptides from the autoantigens HC gp-39 and human CII to antigen-specific MHC-restricted T cell hybridomas. This response required pretreatment of FLS with IFNγ, showed MHC restriction, and was dependent on human class II MHC and murine CD4 for effective antigen presentation. Furthermore, FLS were able to extract and present antigens found within human SF to both the HC gp-39 and human CII T cell hybridomas in an IFNγ-dependent and MHC-restricted manner.

Conclusion. RA FLS can function as APCs and are able to present peptides derived from autoantigens found within joint tissues to activated T cells in vitro. In the context of inflamed synovial tissues, FLS may be an important and hitherto overlooked subset of APCs that could contribute to autoreactive immune responses.

In contrast to the usual functions of fibroblasts, fibroblast-like synoviocytes (FLS) have been hypothesized to be important in joint inflammation and destruction (1–4). In rheumatoid arthritis (RA), FLS adopt an inflammatory phenotype and secrete cytokines, proteases, and other mediators. FLS secrete chemokines that enhance leukocyte recruitment through synovial endothelium (5). The most abundant infiltrating leukocyte is the T lymphocyte, which may come into close proximity to FLS, and interaction between these cells could contribute to RA pathology. When FLS and T cells are cocultured in vitro, activation of both cell types is observed. FLS produce inflammatory mediators such as interleukin-6 (IL-6), IL-8, and prostaglandin E_2 (6), and T cells up-regulate the activation markers CD69 and CD25 (7). The functional consequences of these cocultures mirror the molecular characteristics of RA synovium, which suggests that similar interactions could be occurring within the inflamed RA joint. These experiments were performed without addition of antigen; thus, the bidirectional activation observed was by mechanisms independent of exogenous antigen presentation.

The etiology of RA is still unknown, although several hypotheses have been proposed; however, a strong genetic contribution to RA is well established.

Within the Caucasian population, the most important genetic association is with the major histocompatibility complex (MHC) locus—specifically with HLA–DR4 and other closely related DR alleles (8). One of the RA-associated DR4 alleles, HLA–DRB1*0401, may also be a predictor of disease severity, with homozygous individuals showing worse outcomes (9–12). The RA-associated DR4 alleles all share a common motif near the peptide binding groove at residues 70–74 of the β-chain (13–15). When human HLA–DRB1*0401 is expressed as a transgene in mice, it confers susceptibility to induction of arthritis by immunization with certain autoantigens, even on otherwise genetically resistant backgrounds (16).

Prior work has yielded mixed results regarding the capacity of fibroblasts to function as true antigen-presenting cells (APCs) for MHC-restricted responses to exogenous antigens from pathogens (17–20). The present study sought to evaluate the ability of FLS to function as APCs for specific autoantigens present within the joint and relevant to RA. Mouse T cell hybridomas were employed that are specifically responsive to arthritogenic peptides of human cartilage gp-39 (HC gp-39, YKL-40) or human type II collagen (CII) presented by the human class II MHC allele HLA–DRB1*0401, as measured by production of murine IL-2. These hybridomas were developed from a mouse transgenic for the human class II allele HLA–DR4*0401. Since these T cell hybridomas have been shown to recognize their respective antigens when presented by human dendritic cells or human monocytes that express *0401 (21), they were used to evaluate the APC potential of FLS for arthritogenic autoantigens.

PATIENTS AND METHODS

Fibroblast isolation and culture. All procedures involving specimens obtained from human subjects were performed under protocols approved by the University of Michigan Institutional Review Board. FLS were obtained by collagenase (Worthington, Freehold, NJ) digestion of human synovial tissue obtained at arthroplasty or synovectomy from RA or osteoarthritic (OA) joints. The diagnosis of RA was based upon the presence of at least 4 of the 7 American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria (22). The diagnosis of OA was based upon characteristic clinical and radiographic features and was confirmed by pathologic findings at joint surgery. Cells were maintained in CMRL medium (Invitrogen, San Diego, CA) supplemented with 10% fetal calf serum (FCS; Atlanta Biological, Atlanta, GA), 2 mM glutamine (Cambrex, Walkersville, MD), 50 units/ml penicillin (Cambrex), and

50 μg/ml streptomycin (Cambrex). FLS were used after passage 4 from primary cultures.

T cell hybridoma generation and culture. T cell hybridomas specific for a 13-amino-acid peptide of the arthritogenic HC gp-39 (263–275) and a 15-amino-acid peptide from human CII proteins (259–273) were developed and characterized as previously described (21). T cell hybridomas were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% FCS, 2 mM glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 0.6 mM sodium pyruvate (Cambrex), 1 mM HEPES (Cambrex), and 0.055 mM β-mercaptoethanol (Invitrogen).

Antigens. Peptide antigens RSFTLASSETGVG and GIAGFKGEQGPKGEP, corresponding to HC gp-39 263–275 and human CII 259–273, respectively, were synthesized by the University of Michigan Protein Core. Synovial fluid (SF) was obtained at therapeutic arthrocentesis, centrifuged to remove cells, and stored at −80°C for subsequent use. For testing of responses to SF antigens, FLS were loaded with 50%/50% SF/medium (for the HC gp-39) or with 20%/80% SF/medium (for the human CII hybridoma) as an antigen source. FLS were incubated with antigens for 3–7 days before addition of T cell hybridomas. Antigen incubation times <3 days resulted in suboptimal antigen presentation.

FLS and T cell hybridoma coculture. FLS were cultured with CMRL medium in 24-well plates at 10,000/well and were allowed to adhere for 48 hours. FLS were then stimulated with 1,000 units/ml interferon-γ (IFNγ) for an additional 48 hours to reinduce class II MHC, which is expressed in vivo by FLS. Four days after initial plating, the medium was changed, and new medium containing 1,000 units/ml of IFNγ and 10 μg/ml of peptide antigen (HC gp-39 or human CII) was added for another 3 days. Seven days after initial plating, the medium was changed to T cell hybridoma medium, containing HC gp-39 or human CII peptide at 10 μg/ml. T hybridoma cells (200,000) were added while maintaining antigen concentration at 10 μg/ml. T cells and FLS were allowed to interact for 3 days before plates were frozen and thawed. Plates were spun down, and supernatants were harvested.

Mouse IL-2 enzyme-linked immunosorbent assay (ELISA). ELISAs were performed using OptEIA Mouse IL-2 kits (BD Biosciences, San Jose, CA). The manufacturer's protocols were followed.

MHC typing. DNA was isolated with the DNeasy Tissue Kit (Qiagen, Chatsworth, CA). MHC analysis used the HLA–DR Typing Tray and DR 4T SSP Unitray (both from Pel-Freez, Rogers, AR) to identify the presence of a DR4 allele and the subtype of DR4, respectively. The manufacturer's protocols were observed.

Blocking antibodies. For blocking assays, T cell hybridomas were incubated, before being cocultured with FLS, in medium containing 10 μg/ml anti-CD4, anti-CD11a, anti-CD2, or rat IgG. FLS were exposed to medium containing 10 μg/ml anti–class II MHC, anti-CD54, anti-CD58, or mouse IgG before the addition of T cell hybridomas. When cells were cocultured, additional antibodies were added to maintain a final antibody concentration of 10 μg/ml.

HC gp-39 ELISA. ELISA for HC gp-39 was performed using a YKL-40 ELISA Kit (Quidel, San Diego, CA). The manufacturer's protocols were followed.
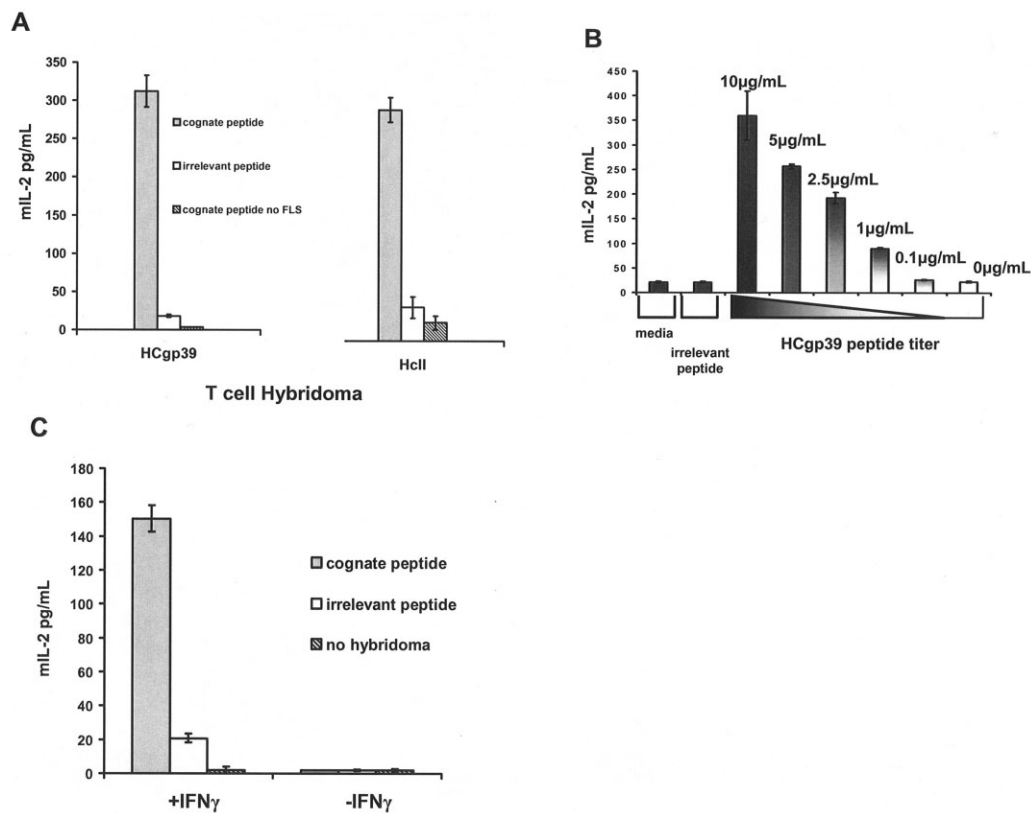
Figure 1. Presentation of arthritogenic peptides to T cell hybridomas by fibroblast-like synoviocytes (FLS). **A,** FLS were plated and treated with interferon-γ (IFNγ). Human cartilage gp-39 (HC gp-39) or human type II collagen (HcII) peptides were pulsed onto FLS before they were cocultured with T cell hybridomas. Mouse interleukin-2 (mIL-2) in culture supernatants from HC gp-39– or human type II collagen–specific T cell hybridomas was measured by enzyme-linked immunosorbent assay. Values are the mean from 1 experiment representative of a total of 5 experiments. Error bars represent the range of the 95% confidence intervals. **B,** FLS were pulsed with decreasing concentrations of HC gp-39 peptide before coculture with the HC gp-39–specific hybridoma. Values are the mean from 1 experiment representative of a total of 2 experiments. Error bars represent the range of the 95% confidence intervals. **C,** FLS received medium either containing or devoid of IFNγ before being pulsed with peptide and cultured with the HC gp-39–specific hybridoma. Values are the mean from 1 experiment representative of a total of 5 experiments. Error bars represent the range of the 95% confidence intervals.

RESULTS

Ability of FLS to present HC gp-39 and human CII peptides. To assess the APC function of FLS, these cells were treated with IFNγ, loaded with immunodominant peptides from HC gp-39 or human CII, and cocultured with the HC gp-39– or human CII–specific T cell hybridoma. For a control, the peptide and T cell hybridoma pairs were mismatched (FLS + HC gp-39 peptide + human CII hybridoma, or FLS + human CII peptide + HC gp-39 hybridoma). These mismatched peptide pairs are subsequently referred to as irrelevant peptide controls. The supernatants from these cocultures were evaluated for murine IL-2 as a measure of T cell stimulation due to FLS presentation of peptide antigen. FLS cocultured with the HC gp-39– or human CII–specific T cell hybridoma cells along with the cognate peptide induced significant release of IL-2 from the hybridomas (Figure 1A). When irrelevant peptide was presented, IL-2 production was greatly reduced. Hybridomas cultured with cognate peptide in the absence of FLS produced very little or no IL-2, indicating the requirement for an APC.

To explore the effects of antigen concentration on the ability of FLS to activate T cell hybridomas, FLS were loaded with various concentrations of peptide antigen, ranging from 0.1 μg/ml to 10 μg/ml, and used as APCs for T cell hybridomas. IL-2 production by the T cell hybridomas increased with the antigen concentration (Figure 1B).
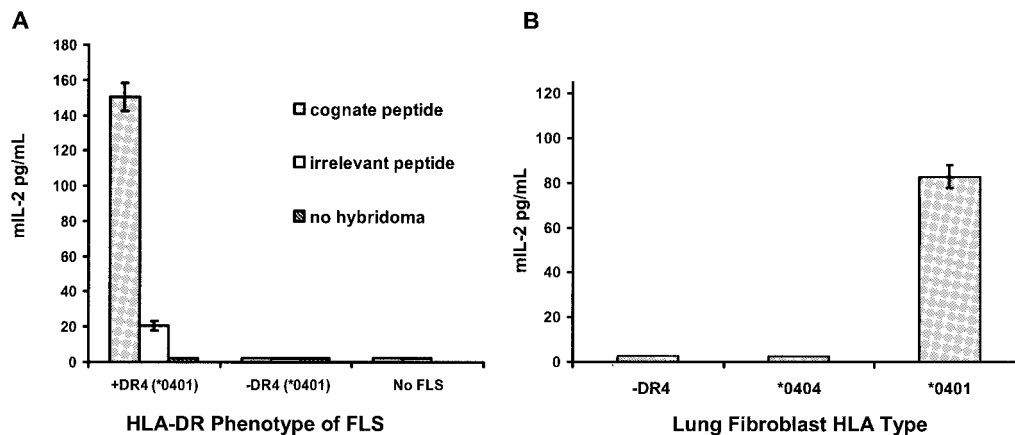
Figure 2. Major histocompatibility complex restriction of antigen presentation by FLS and lung fibroblasts. **A,** HLA–DRB1*0401–positive and –negative FLS lines were used to present peptide antigen to an HC gp-39 T cell hybridoma. Values are the mean from 1 experiment representative of a total of 3 experiments. Error bars represent the range of the 95% confidence intervals. **B,** Arthritogenic peptide antigen presentation by fibroblasts is restricted to HLA–DRB1*0401. Lung fibroblasts expressing the rheumatoid arthritis–associated DR4 subtype alleles *0401 or *0404 were pulsed with HC gp-39 peptide and cocultured with the HC gp-39 T cell hybridoma. IL-2 was measured in supernatants by enzyme-linked immunosorbent assay. Values are the mean from 1 experiment representative of a total of 3 experiments performed in duplicate. Error bars represent the range of the 95% confidence interval. See Figure 1 for definitions.

Dependence on IFNγ of FLS APC function. We assessed the requirement of IFNγ pretreatment for effective APC function by FLS. FLS, with or without IFNγ pretreatment, were cultured in medium containing HC gp-39 peptide antigen and used as APCs for the HC gp-39 T cell hybridoma (Figure 1C). IFNγ pretreatment was necessary to reinduce expression of class II MHC found in vivo on these FLS, which had been passaged in vitro. IFNγ-treated FLS were able to present antigen to T cell hybridomas, but FLS cultured without IFNγ did not function as APCs. The IFNγ dependence of FLS APC function is consistent with class II MHC–dependent antigen presentation and recognition.

Class II MHC restriction of fibroblast APC function. Not all FLS lines were able to function as APCs, even with IFNγ stimulation. It was hypothesized that lack of APC function by FLS was due to the MHC restriction of the T cell hybridoma response. Chromosomal DNA from various FLS lines, as well as from fibroblast lines from other tissue sources, were harvested and screened for HLA type by polymerase chain reaction. Using HLA–DRB1*0401–positive and DR4-negative FLS lines as APCs for peptide antigens, we found that only *0401-positive cell lines could present antigen to the HC gp-39 T cell hybridoma (Figure 2A). When a DR4-negative cell line was used as an APC, no IL-2 was produced by the T cell hybridoma.

We next sought to assess the DR4 subtype specificity of antigen presentation and also the antigen presentation potential of fibroblasts other than FLS. To address these issues, MHC-typed lung fibroblasts cultured from interstitial pneumonia biopsy samples were treated with IFNγ, loaded with cognate antigen or irrelevant antigen, and used as APCs. Similar to FLS, lung fibroblasts were able to load peptide antigen and present it to the HC gp-39 T cell hybridoma (Figure 2B). Comparing *0401-positive, *0404-positive, and DR4-negative lung fibroblast lines, this system showed stringent specificity for antigen recognition only in the context of *0401. Even the *0404 allele, which also contains the shared epitope and is associated with RA, did not function as an effective activation signal for the T cell hybridoma. The ability of fibroblasts to function as APCs is therefore not unique to fibroblasts from a synovial source, and antigen presentation by fibroblasts of any tissue follows strict MHC restriction.

Table 1 summarizes the results of HLA–DR typing of fibroblasts from various tissue sources and assessment of APC function of the fibroblasts used as APCs for the HC gp-39 T cell hybridoma. The ability of fibroblasts to act as APCs corresponds strictly to possession of the correct class II MHC subtype, specifically HLA–DRB1*0401, irrespective of the tissue source. Notably, DRB1*0403- and *0404-expressing fibroblasts did not function as APCs for the HC gp-39 T cell hybridoma. Similar to professional APCs, fibroblast APCs must express the class II MHC allele with which T cells were educated.

Table 1. Results of HLA–DR typing of fibroblasts from various tissue sources and assessment of APC function of the fibroblasts used as APCs for the human cartilage gp-39 T cell hybridoma*

Cell type, cell line	DR4 subtype	APC function
Synovial		
04M098	*0401	+
04F115	*0401	+
RWS	*0401	+
CLC	*0401	+
04F096	*0401	+
RA35	None	−
RA77	None	−
Lung		
100B	*0401	+
89A	*0404	−
103B	None	−
Skin		
R1652	*0403	−

* APC = antigen-presenting cell.

Dependence of APC function on class II MHC and CD4. Given that IFNγ is required to achieve effective APC function of FLS, and given that class II MHC restriction is displayed by T cell hybridomas, the roles of class II MHC and other structures important to T cell activation were further evaluated. IFNγ stimulation up-regulates many proteins important for antigen presentation. We sought to isolate those critical to FLS–T cell interactions. Blocking antibodies were added to cocultures of antigen-loaded FLS and T cell hybridomas, including human class II MHC, CD54, and CD58 on the FLS surface and murine CD4, CD2, and CD11a on the T cell surface. Blockade of human class II MHC or murine CD4 by antibody abolished the FLS activation of the HC gp-39 T cell hybridoma (Figure 3), and thus further demonstrated the importance of a functional peptide–MHC complex. Interference with human CD54 adhesion yielded less robust effects, and blockade of murine CD11a did not cause a similar reduction in IL-2 production. Thus, it is unclear at this time whether a CD54–CD11a interaction is required for FLS to present antigen to T cell hybridomas. A CD58–CD2 interaction also does not seem to be required for FLS APC function in this system.

Ability of FLS to present autoantigens from SF. To use a source of antigen more biologically relevant than synthetic peptides, human SF from RA and OA patients was added to cultures of FLS. SF was collected from patients undergoing therapeutic arthrocentesis. Collected SF was centrifuged at 2,000 revolutions per minute for 30 minutes to pellet synovial cells. The supernatant was used as an antigen source after being diluted to 20% in medium. FLS were able to extract human CII antigens from some SF and activate the human CII T cell hybridoma (Figure 4A). One SF sample was able to elicit IL-2 responses that were comparable with 10 μg/ml of peptide antigen (RA SF8). Other SF samples (RA SF6 and OA SF3) were presented by FLS, but were not as potent as 10 μg/ml of peptide antigen. FLS were unable to present antigen from some SF samples. None of the SF samples tested activated the human CII hybridoma in the absence of FLS; however, 1 SF sample did activate the HC gp-39 hybridoma minimally (data not shown).

To assess whether the variability in the ability of different SF samples to function as a source of antigen reflected the antigen concentration present within the fluid, various SF samples were presented to the HC gp-39 T cell hybridoma by FLS. The HC gp-39 content within these SF samples was measured by ELISA, and these SF samples were then loaded onto FLS for antigen presentation. The HC gp-39 concentration was lower in SF than the amount of peptide used for antigen presentation. SF HC gp-39 concentrations ranged from 1.19 μg/ml to 3.10 μg/ml (Figure 4B). FLS were able to extract HC gp-39 from some SF samples and activate T cell hybridomas (Figure 4B). Functional HC gp-39 antigen was present in both RA and OA SF, similar to human CII antigen, as indicated by induction of IL-2. However, not all SF samples allowed FLS to activate hybridomas, even when HC gp-39 antigen was detect-
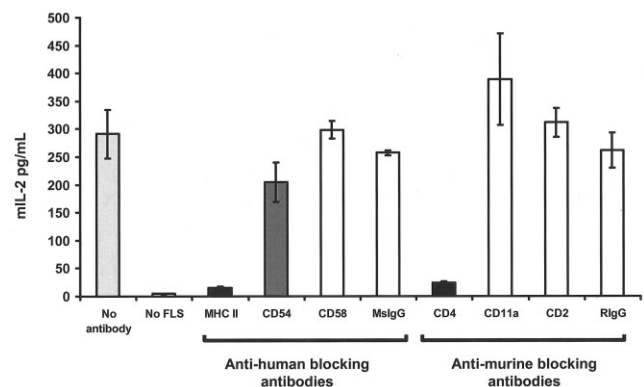

Figure 3. Class II major histocompatibility complex (MHC II) and CD4 dependency of the antigen-presenting cell function of FLS for peptide antigen responses of T cell hybridomas. Blocking antibodies to cell surface structures were added to cocultures of FLS and the HC gp-39 T cell hybridoma. Mouse IL-2 was measured by enzyme-linked immunosorbent assay. Values are the mean from 1 experiment representative of a total of 2 experiments performed in duplicate. Error bars represent the range of the 95% confidence intervals. MsIgG = mouse IgG; RIgG = rat IgG (see Figure 1 for other definitions).
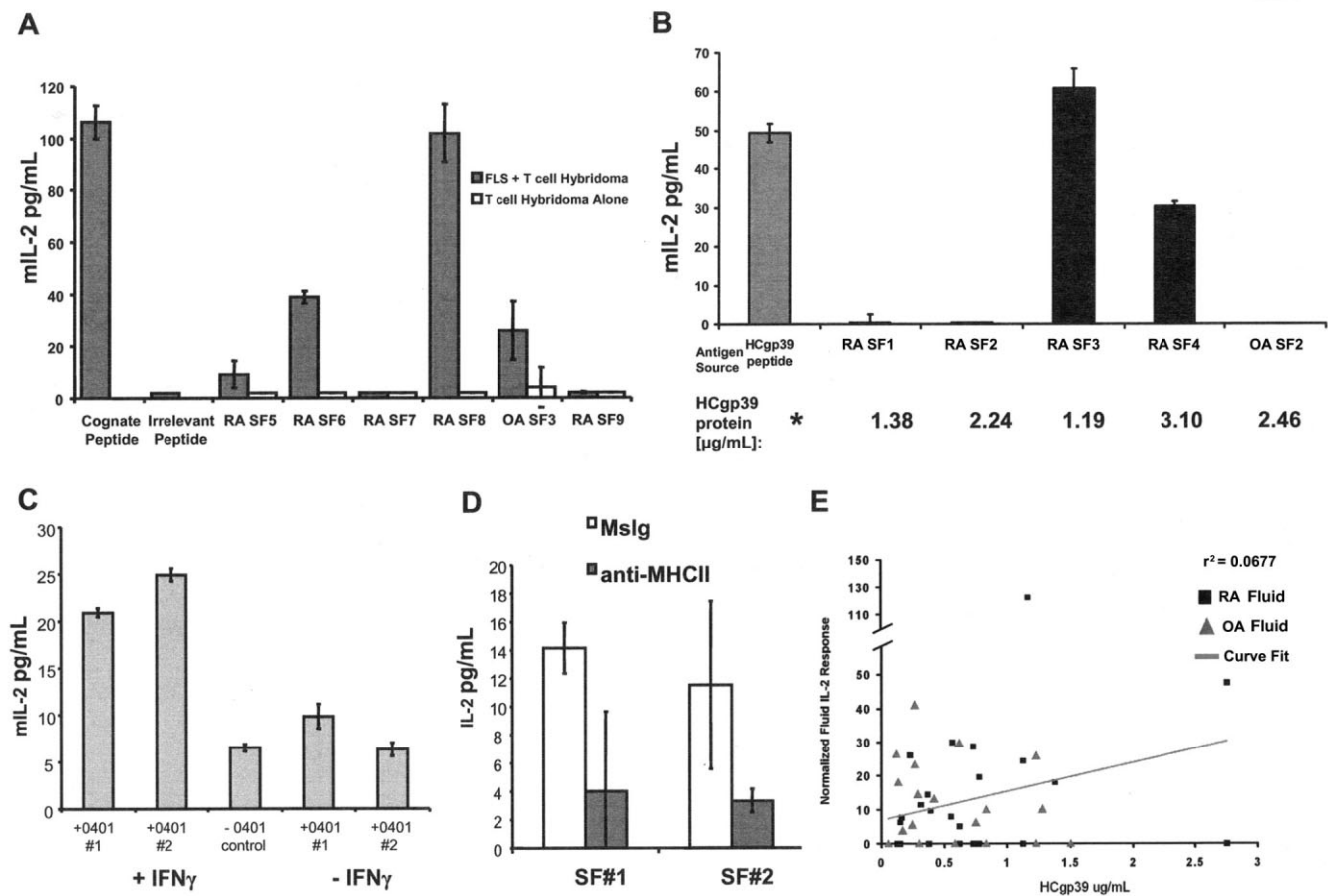
Figure 4. Ability of FLS to extract antigen from synovial fluid (SF) and present it to HC gp-39 and human type II collagen (human CII) T cell hybridomas. **A,** SF samples from rheumatoid arthritis (RA) and osteoarthritis (OA) patients were diluted to 20% in medium and loaded onto FLS before coculture with the human CII–specific T cell hybridoma. **B,** FLS were loaded with RA or OA SF samples diluted to 50% in medium before coculture with the HC gp-39–specific T cell hybridoma. The HC gp-39 concentration within the SF was measured by enzyme-linked immunosorbent assay and is indicated below the x-axis. **C,** SF was diluted to 20% in medium and loaded onto FLS before coculture with human CII–specific T cell hybridomas. Two *0401-positive FLS lines and one *0401-negative FLS line were used as antigen-presenting cells; the *0401-positive FLS lines were cultured with or without IFNγ. **D,** Two different SF samples were loaded onto FLS before coculture with the human CII–specific T cell hybridoma. Antigen presentation was inhibited by 10 μg/ml of mouse anti-human class II major histocompatibility complex (anti-MHCII). Control antibody was mouse immunoglobulin (MsIg). Values in **A–D** represent the mean. Error bars in **A–D** represent the range of the 95% confidence intervals. **E,** OA or RA SF was loaded onto FLS and presented to the HC gp-39 T cell hybridoma. The resultant IL-2 production was represented as a percentage of the IL-2 produced from 10 μg/ml of HC gp-39 peptide and plotted on the y-axis. The x-axis displays the concentration of the HC gp-39 antigen contained within SF. Linear regression analysis yielded an r² value of 0.0677. See Figure 1 for other definitions.

able by ELISA at concentrations similar to those in functionally presentable SF samples. One SF sample activated the HC gp-39 hybridoma in the absence of FLS and was excluded (data not shown).

Given the variability of antigen presentation using SF, we sought to ensure that antigen presentation under these conditions required class II MHC expression, restriction, and dependence. To assess the requirement for class II MHC expression and restriction, one *0401-negative and two *0401-positive FLS lines were

used to present SF to the human CII hybridoma. In a parallel experiment, the *0401-positive FLS lines were not prestimulated with IFNγ (Figure 4C). The same SF sample was used for all conditions. In the absence of IFNγ stimulation, neither *0401-positive FLS line presented antigens from SF to the T cell hybridoma, and only basal levels of IL-2 were detectable. After IFNγ stimulation, the *0401-positive FLS lines were able to present antigen derived from SF to the human CII hybridoma. The *0401-negative FLS line was unable to

present SF antigens to the T cell hybridoma even after IFNγ stimulation, and only basal levels of IL-2 were detectable (similar to no IFNγ treatment) from these cultures. IFNγ dependence and class II MHC restriction provide strong evidence that antigen presentation of SF by FLS is MHC dependent. To further prove the class II MHC dependence of SF presentation, we employed class II MHC blocking studies. Using 2 different SF samples as antigen sources for FLS (Figure 4D), antibody to class II MHC specifically inhibited effective APC function by FLS for both SF samples.

The lack of correlation between antigen concentration in SF and the level of IL-2 produced by the HC gp-39 T cell hybridoma contrasted with previous results (Figure 1B), which showed that the level of IL-2 produced by T cell hybridomas correlated with the concentration of peptide antigen presented. To explore this discrepancy, we pooled the data from several experiments that measured the antigen concentration of SF and the ability of FLS to present HC gp-39 from SF to the HC gp-39 hybridoma. For an internal control, the resultant IL-2 response from these experiments was normalized as a percentage of the IL-2 response elicited by loading the same FLS lines with 10 μg/ml of synthetic peptide. This normalized response was plotted against the HC gp-39 concentration in the SF as measured by ELISA. A close correlation between the HC gp-39 concentration in SF and the amount of IL-2 produced by the HC gp-39–specific T cell hybridoma was not observed (Figure 4E). Curve fitting analysis yielded a very low r^2 value of 0.0677. We further evaluated some SF samples that were not presentable by FLS, by adding 10 μg/ml of HC gp-39 to the fluids before coculture with the HC gp-39 T cell hybridoma. These SF samples exerted an inhibitory effect on peptide antigen presentation (data not shown). This result implies that in addition to the presence of specific antigen, other as-yet-uncharacterized factors in some SF samples regulate the capacity of FLS to function as APCs.

DISCUSSION

Presentation of autoantigens to T cells in synovial tissue could be very important in the initiation and perpetuation of inflammatory arthritis. The RA-associated MHC alleles possess a common sequence motif referred to as the "shared epitope" (13). Structural analysis of the shared epitope region shows that it is positioned near the MHC peptide binding groove (15); thus, it influences bound peptides or affects interactions with T cell receptors (TCRs) (14). This suggests that antigen presentation may be an important pathogenic mechanism in RA.

Prior work has documented T cell–FLS interaction leading to activation of both cell types (6,7,23), but previous data regarding fibroblasts as APCs have been mixed. Investigators in early studies using dermal fibroblasts observed that they were poor generators of allogeneic responses (17). This defect was not due to inadequate expression of class II MHC, but rather to the lack of an accessory molecule that could be provided by conventional APCs. However, it was noted that dermal fibroblasts could stimulate previously activated alloreactive T cells. Expanding on this work, the capacity of dermal fibroblasts to function in antigen presentation was evaluated. Dermal fibroblasts were able to process exogenous antigen, but did not function well as APCs without accessory cell help (18,24). In both of those studies, IFNγ was used to induce class II MHC, but human autoantigens were not evaluated. These previous studies do document fibroblast expression of functional class II MHC.

FLS of RA synovium express high levels of class II MHC in vivo and ex vivo (25), suggesting that the potential exists for antigen presentation by FLS in RA. Previous work indicated that FLS can process and present bacterial antigens to T cell clones via a class II MHC–restricted mechanism (19). FLS can also present bacterial superantigens to polyclonal peripheral blood lymphocytes, activating a significant subset of T cells. However, these reports focus on exogenous antigens from pathogens. Our current study has documented the ability of FLS to function as APCs for human autoantigenic peptides and endogenous human proteins present in SF, indicating that these interactions could be occurring in vivo within an inflamed joint, in the absence of infection.

There is also evidence that under some conditions FLS might not activate T cells, but instead induce anergy, in experiments that assessed the APC and allostimulatory functions of FLS (26). In these studies, FLS were able to load antigen onto class II MHC, but allogeneic responses depended upon the addition of accessory cells expressing CD80, and blockade of CD80 abolished the response (i.e., FLS are poor allogeneic stimulators, similar to dermal fibroblasts [17]). When FLS without accessory cells were cultured with T cells, the T cells adopted a phenotype resembling anergy, characterized by up-regulation of CD25, reduced proliferation, and reconstitution of proliferation by exogenous IL-2. This result implies that FLS cause anergy due to a lack of costimulatory molecules, but that bystander cells

expressing costimulatory molecules could overcome this. The potential for accessory costimulation exists abundantly within RA synovium due to the close proximity of FLS to B cells, macrophages, and dendritic cells, as well as other T cells.

The evidence that FLS can express functional class II MHC is strong, but the function of FLS MHC expression has not been thoroughly explored. Work in transgenic mice suggests that the specific class II MHC allele subtype has a striking effect on T cell polarization. Evaluation of the T cells from DR4-transgenic mice reveals functional differences between RA-associated and non–RA-associated alleles. T cells from *0401-transgenic mice, possessing the RA-associated allele, differ from T cells from *0402-transgenic mice, which carry a non–RA-associated allele, by their cytokine profile after antigen stimulation. Mice transgenic for *0401 show a skew toward a Th1-mediated immune response and make greater levels of IFNγ and tumor necrosis factor α (TNFα) after stimulation with antigen compared with *0402-transgenic mice (20).

A possible reason for differing T cell responses to antigens presented by different DR4 alleles is that the peptide binding repertoire of RA-associated DR4 alleles is distinct from that of other DR4 alleles. HLA–DRB1*0401 presents immunodominant peptides from the autoantigens HC gp-39 and human CII, which are peptides distinct from those presented by non-RA DR4 alleles (20). Perhaps the presentation of a unique panel of peptides by RA-associated MHC activates a distinct set of T cells in the periphery and educates a corresponding set of T cells in the thymus, thus producing the predisposition to autoimmunity (27,28).

Even though a definitive autoantigen (or autoantigens) has yet to be consistently identified in this process of T cell development and activation in RA, HC gp-39 and human CII are plausible candidates. These autoantigens are found within cartilage and synovial tissues and are arthritogenic, able to induce inflammatory arthritis in susceptible strains of rodents (29). HC gp-39 is made by chondrocytes and macrophages, 2 cell types that are found in RA joints. Elevated serum levels of HC gp-39 appear to correlate with increased RA disease activity (30–32). Peripheral blood mononuclear cells from RA patients show an increased proliferative response to HC gp-39 antigen compared with those from healthy controls (33). The prevalence of HC gp-39–responsive T cells in peripheral blood of RA patients was similar to that seen in controls in 1 study (34), but RA T cells produced increased IFNγ in response to HC gp-39, whereas controls showed an IL-10 response,

indicating that there is a skew toward inflammation in RA patients, while controls show a regulatory response (35). Histologic studies have even identified HC gp-39 complexed with HLA–DRB1*0401 on APCs within human RA synovial tissue sections (36). These observations support the idea that HC gp-39–responsive T cell clones might contribute to RA pathogenesis or joint inflammation.

Like HC gp-39, human CII is a human autoantigen, also used to induce arthritis in mice as a model of RA (37). It is only found within cartilage and synovial fluid or tissue. Although autoantibodies to human CII and human CII–responsive T cell clones are not specific for RA (38), T cell clones in RA patients have been identified that have a TCR repertoire similar to that of human CII–expanded T cells (39). There is also evidence that T cell responses to altered forms of CII are important in RA (40). Furthermore, cocultures of human CII–reactive T cells with FLS increased the production of IFNγ, IL-17, TNFα, IL-15, and IL-18 (23). These studies suggest that T cell responses are altered in RA patients such that responses to antigens might be skewed toward a proinflammatory response centered around HC gp-39 and/or human CII antigens, even when measurable T cell proliferative responses are not remarkably elevated.

In the current study, HLA–DRB1*0401–positive fibroblasts from different tissue sources were able to present immunodominant peptides from the arthritogenic proteins HC gp-39 and human CII to antigen-specific T cell hybridomas. The key requirement appears to be the expression of the correct class II MHC allele. Since T cell hybridomas represent previously activated T cells, minimal costimulation may be needed for reactivation. Nonetheless, any activation of the T cell hybridoma indicates that a functional peptide–class II MHC complex is present on the FLS. If irrelevant peptide or a class II MHC mismatch is present, no T cell stimulation occurs. The significance of other structural interactions outside of MHC–TCR is not yet clear. The CD54–CD11a and CD58–CD2 interactions are often important for professional APC interaction with T cells. Inhibiting these interactions did not substantially disrupt FLS APC function. Perhaps cross-species limitations of some costimulatory receptor–ligand interactions and the relative lack of dependence of hybridomas on costimulatory signals minimize the roles of these molecules in the system that we used.

FLS are also able to extract antigens from SF and present the antigens to both HC gp-39 and human CII T cell hybridomas. The exact nature of the antigenic

material in SF is still unknown. It may include predigested peptide fragments and/or intact proteins, such as HC gp-39. Whether these antigens are passively loaded onto FLS or are ingested and processed by FLS remains to be discovered, and it is possible that both processes can occur. In the current experiments, it was important to exclude possible mechanisms of T cell–FLS interaction that did not involve antigen presentation, and to prove that FLS induction of the IL-2 by T cell hybridomas was through antigen presentation. Thus, activation of T cell hybridomas by FLS, as measured by IL-2, showed MHC restriction, was antigen specific, required IFNγ, and was dependent on functional class II MHC. Moreover, SF from patients with RA or OA almost never induced secretion of IL-2 by hybridomas in the absence of FLS.

In the present study, we did not find a correlation between the concentration of HC gp-39 in SF and the ability of FLS to present antigens to the HC gp-39 T cell hybridoma. The specificity of the HC gp-39 ELISA regarding which region of the protein is recognized by antibodies is unknown; the recognized portion could be inconsequential to antigen presentation. Moreover, it is possible that there are HC gp-39 degradation products present in SF that were not recognized by the HC gp-39 ELISA. These fragmented portions of HC gp-39 could result in a "functionally" high concentration of HC gp-39, while leaving the measurable HC gp-39 low. Conversely, the measurable HC gp-39 could be in relative abundance, while the portions responsible for antigen presentation are reduced, leading to high measured concentrations of HC gp-39 with little stimulatory effects on the hybridoma.

In addition to uncertain protein concentration, SF also contains many factors that could affect antigen presentation, enhancing or inhibiting it. Inflammatory and inhibitory cytokines (e.g., IL-1 and IL-10) are present in SF and may affect immune cell function. Failure of FLS to present recombinant peptides in the presence of certain SF samples supports the concept of inhibitory factors within those SF samples (data not shown). It is also notable that while professional APCs can effectively present HC gp-39 and human CII antigens with overnight incubation with antigen and 20-hour coculture times with T cells, the kinetics are different for FLS as APCs. FLS require prolonged incubation with antigen and longer coculture times with T cells. This suggests that FLS might not process and/or present antigen as efficiently as their professional counterparts, or that they might use entirely different mechanisms to achieve the same ends. Given the chronicity of RA, the prolonged time needed for FLS–T cell interactions would not be an obstacle to the occurrence of such interactions in vivo. These issues will require further analysis in order to identify additional factors that govern FLS APC function in vitro and in vivo.

FLS exist under unique conditions that provide mechanisms for stimulation of T cells specific to arthritogenic autoantigens. FLS express high levels of class II MHC in RA synovium and are chronically exposed to autoantigens present within SF. Our studies indicate that FLS can express a functional autoantigen–class II MHC complex. In the context of an inflamed RA joint, FLS could take up antigen, display antigenic peptides on MHC, and activate T cells in an antigen-dependent mechanism as suggested by findings of our in vitro studies. However, the data in this report do not establish that FLS can take up and process antigens with the same efficiency as professional APCs. Nevertheless, the role of nonclassic APCs (such as fibroblasts) as participants in autoimmunity deserves further consideration.

AUTHOR CONTRIBUTIONS

Dr. Fox had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study design. Tran, Davis, Lundy, Kovats, Fox.
Acquisition of data. Tran, Davis, Tesmer, Endres, Motyl, Smuda, Somers, Chung, Urquhart, Lundy, Fox.
Analysis and interpretation of data. Tran, Davis, Motyl, Lundy, Fox.
Manuscript preparation. Tran, Endres, Lundy, Kovats, Fox.
Statistical analysis. Tran.
Manuscript review. Somers, Chung, Urquhart.

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