Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9131–9135, October 1992
Immunology

Expression of myosin–class II major histocompatibility complexes in the normal myocardium occurs before induction of autoimmune myocarditis

(self-proteins/dendritic cell/antigen presentation)

STACY C. SMITH*† AND PAUL M. ALLEN‡

Division of Cardiology, Departments of *Internal Medicine and ‡Pathology, Washington University School of Medicine, 660 South Euclid, St. Louis, MO 63110

Communicated by Emil R. Unanue, June 25, 1992

ABSTRACT **Determining how an autoimmune response is initiated is essential to understanding the mechanisms of autoimmunity. Self-reactive T cells, self-protein, and a failure of tolerance to that self-protein are all involved in the pathogenesis of autoimmune disease; yet it is not clear how self-reactive T cells find the target self-protein to initiate an autoimmune response. Although a variety of self-proteins have been shown to be presented on both class I and class II major histocompatibility complex (MHC) molecules, the relationship of these self-proteins to autoimmune disease has not been established. To explore this further, we generated a T-cell hybridoma that recognizes mouse cardiac myosin, the self-protein that induces murine autoimmune myocarditis. Using this hybridoma as a probe to detect myosin–class II MHC complexes, we isolated a class II MHC$^+$/CD45$^+$ residential antigen-presenting cell (APC) population directly from the hearts of normal mice and looked for evidence of endogenous processing of cardiac myosin by these APC. In this report we show that myosin–class II MHC complexes are found on residential APC in the normal mouse heart. Induction of autoimmune myocarditis increased the expression of myosin–class II MHC in the heart and enhanced their APC functions. This result is a direct demonstration that epitopes of a self-antigen involved in initiating an autoimmune disease are endogenously processed and presented within the target organ.**

It is vital that the immune system distinguish between self-proteins and foreign protein antigens to elicit an effective immune response. Precisely how this discrimination is made is not completely understood, but negative selection (1–7), clonal anergy (8), peripheral tolerance (9), and down-regulation of self-reactive T-cell receptors (10) are all mechanisms shown to function *in vivo* to sustain tolerance to self-proteins. Work by several investigators has firmly established that self-proteins are processed and presented by antigen-presenting cells (APC) (11–15). Wekerle *et al.* (16) reported that Schwann cells could process the self-protein myelin basic protein, known to initiate experimental allergic encephalomyelitis, and present it to myelin basic protein-reactive T cells, but this occurs only if the Schwann cells are first treated with interferon γ *in vitro*. Recently, endogenously processed self-peptides have been eluted from both major histocompatibility complex (MHC) class I and class II molecules, sequenced, and identified (17–21); these self-peptides represent a spectrum of nuclear, cytoplasmic, and membrane-bound self-proteins. Thus, it is clear that many self-proteins are endogenously processed and complexed with MHC class I or class II molecules. However, none of the currently identified self-proteins known to be endogenously processed by APC *in vivo* has been implicated in an autoimmune disease.

Several models of autoimmune disease use a purified self-protein emulsified in complete Freund's adjuvant (CFA) to induce an autoimmune response in susceptible mice. Examples include experimental allergic encephalomyelitis (22), experimental autoimmune uveitis (23), collagen-induced arthritis (24), experimental autoimmune thyroiditis (25), and myosin-induced myocarditis (26). Because these immunizing self-proteins induce an organ-specific autoimmune disease, we reasoned that self-protein–class II MHC complexes might exist endogenously within the target tissue. A question central to understanding the induction of autoimmune disease in these models is whether self-protein–class II MHC complexes exist before the start of disease or only after target-organ destruction began. To explore this question, we used the model of myosin-induced myocarditis to determine whether self-peptides of cardiac myosin were endogenously presented on class II MHC molecules within the heart. We have shown (27) that CD4$^+$ T cells are critical for inducing this disease and that nonimmunogenic MHC class II-binding peptides can block initiation of cardiac inflammation. Therefore, we focused our attention on the class II-restricted responses. We generated cardiac myosin-specific T-cell hybridomas to probe APC isolated directly from mouse hearts. In this report we demonstrate that myosin–class II MHC complexes are endogenously presented on residential cardiac APC and that these APC are fully capable of processing proteins and stimulating T-cell hybridomas.

MATERIALS AND METHODS

Animals. BALB/cByJ, DBA/2J, A/J, and B10.D2 male mice were obtained from The Jackson Laboratory, housed in microisolator cages (Allentown Caging, Allentown, NJ) and fed autoclaved chow and sterile water ad libitum. Severe combined immunodeficient (SCID) and C.B-17 male mice were obtained from E. Unanue (Washington University). All mice were 2–4 mo of age at the time of experiment. CD1 mice, Lewis rats, and New Zealand White rabbits were used for cardiac myosin purification.

Antigens. Mouse, rat, and rabbit cardiac myosin and mouse skeletal-muscle myosin were purified according to the method of Shiverick *et al.* (28). Actin, troponin, tropomyosin, and ovalbumin were obtained from Sigma. Cyanogen bromide (CNBr) digests of myosin, actin, troponin, and tropomyosin were prepared by incubation at 25°C overnight with a 500-fold molar excess of CNBr in 70% (vol/vol) formic acid,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: APC, antigen-presenting cell(s); MHC, major histocompatibility complex; CFA, complete Freund's adjuvant; IL-2, interleukin 2; PEC, peptone-elicited macrophages.

†To whom reprint requests should be addressed.

as described (35). Hemoglobin was prepared from hemolysates of freshly obtained BALB/cByJ red blood cells, as described (11), and the peptide $Hb^{\beta dmin}$ (64-76) was synthesized by using a DuPont RaMPs system, as published (11). A cell-free extract of heart protein was prepared by removing an aliquot from the digestion mixture used to isolate cardiac APC (described below) before cell separation on Percoll gradients. After centrifugation at 1200 × *g* to remove cells, total protein concentration was determined by using the Bradford assay (Bio-Rad).

Generation of T-Cell Hybridomas. C.B-17 mice were immunized with 200 μg of purified mouse cardiac myosin (27) emulsified in CFA (Difco) on days 0 and 7. On day 21, spleens were removed, a single-cell suspension was prepared, and 5 × 10^6 cells per ml were cultured at 37°C for 7 days in Dulbecco's modified Eagle's medium (DMEM) (GIBCO)/5% fetal bovine serum/2 mM glutamine/10 mM Hepes/1 mM pyruvate/0.1 mM nonessential amino acids/gentamicin at 50 μg/ml/5 × 10^{-5} M 2-mercaptoethanol/purified cardiac myosin at 5 μg/ml. Next, irradiated BALB/cByJ spleen cells at 25 × 10^6 cells per ml and cardiac myosin at 5 μg/ml were added, and after 3 days of culture, recombinant interleukin 2 (IL-2) (35 units per ml) was added for an additional 3 days. The live cells were then recovered by using Ficoll-Hypaque (Pharmacia) and fused to BW5147 T-cell-receptor $\alpha^-\beta^-$ cells according to standard protocol (29). IL-2-producing, cardiac myosin-reactive hybrids were identified with IL-2-dependent CTLL-2 cells (30) as described (11). A CNBr digest of myosin was used as the antigen source because native myosin is relatively insoluble in culture medium and toxic at higher concentrations. The antigen-reactive cells were then subcloned twice at limiting dilution to ensure clonality.

Characterization of Hybridomas. Peptone-elicited macrophages (PEC) were used as APC and prepared from mice previously injected with 60 μg of Con A (Sigma) as described (31). The appropriate concentration of test antigen was then added, 1 × 10^5 hybridoma cells were added, and the mixture was incubated overnight at 37°C. IL-2 production was assessed with CTLL-2 cells as described above. MHC restriction was assessed by blocking studies with the $I\text{-}E^{d/k}$-specific monoclonal antibody 14-4-4S and the $I\text{-}A^d$-specific monoclonal antibody MKD6. Both hybridomas were obtained from the American Type Culture Collection.

Induction of Autoimmune Myocarditis. One hundred fifty micrograms of mouse cardiac myosin was emulsified in CFA and injected s.c. on days 0 and 7 along with 500 ng of pertussis toxin (List Biological Laboratories, Campbell, CA) injected i.p. on day 0 only, as described (27).

Isolation of Cardiac APC. Cells were isolated by a modification of the procedure developed by Yamada and Corr (32). Specifically, mice were killed by cervical dislocation, and the heart was immediately perfused via direct left ventricular puncture with 5 ml of ice-cold Ca^{2+}-free Krebs' solution (32)/100 μM chloroquine (Sigma) followed by 2 ml of Ca^{2+}-Hepes (32) solution, also containing collagenase L at 1 mg/ml (Sigma). The hearts were removed, placed on ice, and minced. An additional 2 ml per heart of the Ca^{2+}-Hepes/collagenase/chloroquine (CHCC) solution was added, and the hearts were digested with gentle rocking at 37°C for 15 min. After the tissue was allowed to briefly settle, ≈90% of the supernatant was gently removed and replaced with an equal volume of fresh CHCC; the hearts were digested for 15 min at 37°C. Trypsin VIII (Sigma) was then added to a final concentration of 20 μg/ml, and the tissue was incubated again for 15 min at 37°C. Soybean trypsin inhibitor (Sigma) was added to a final concentration of 40 μg/ml, and a single-cell suspension was prepared by gently and repeatedly forcing the tissue through a syringe. After filtration over Nytex (Tetko, Briarcliff Manor, NY) to remove any remaining tissue clumps, the cells were washed and resuspended in DMEM/10% fetal bovine serum. This mixture was layered over a discontinuous Percoll gradient by using Percoll solutions at densities of 1.06 g/ml and 1.04 g/ml to separate the APC population from the myocytes.

Assessment of T-Cell Hybridoma Reactivity on Cardiac APC. Cardiac APC were isolated as described, and 2 × 10^4 cells per well were plated into 96-well microtiter plates. The myosin-specific hybridoma CDL 7.8.1 was added to the APC at 1 × 10^5 cells per well in a final volume of 200 μl; no exogenous antigen was added. Spleens were also removed from the same animals, a single-cell suspension was prepared, and 3 × 10^5 irradiated (2000 rads; 1 rad = 0.01 Gy) splenocytes per well were plated in a final volume of 200 μl along with 1 × 10^5 hybridoma cells. Control wells for both the cardiac APC and the spleen cells contained CNBr–myosin at 30 μg/ml.

Fluorescence-Activated Cell Sorter (FACS) Analysis. Cardiac APC were incubated on ice for 30 min with fluorescein isothiocyanate (FITC)-conjugated anti-CD45 (I3/2.3) (33) and biotinylated anti-$I\text{-}A^d$ (MKD6) (provided by Kenneth Murphy, Washington University). Cells were also stained with anti-CD11b (M1/70.15.11.5) (American Type Culture Collection) and FITC-conjugated goat anti-rat IgG (Southern Biotechnology Associates, Birmingham, AL). After washing, R-phycoerythrin-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA) was added to the MKD6-stained samples, and the cells were analyzed on a Becton Dickinson FACScan.

RESULTS

Determination of T-Cell Hybrid Specificity. Of the five myosin-reactive hybridomas we obtained, four were $I\text{-}E^d$-restricted, and one was $I\text{-}A^d$-restricted. Hybridoma CDL 7.8.1 was chosen to represent the panel of hybridomas, and the antigen specificity of this hybrid is shown in Fig. 1. This hybridoma reacted to native mouse cardiac myosin but did not react to native rat or rabbit cardiac myosin, mouse skeletal muscle myosin, or the cardiac and skeletal isoforms of actin, troponin, and tropomyosin. Both chloroquine treat-

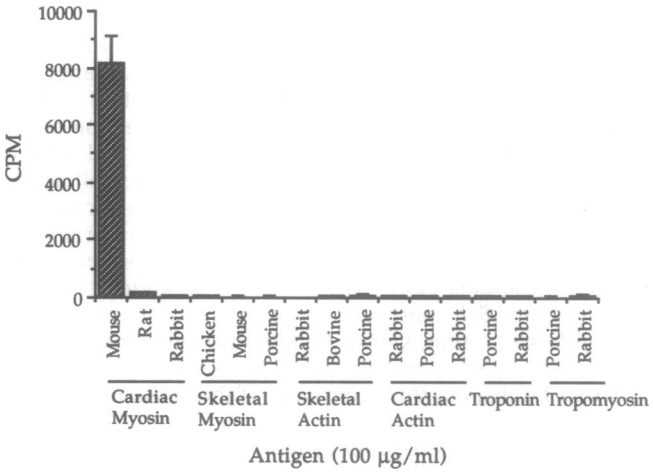

FIG. 1. Stimulation of hybridoma CDL 7.8.1 by cardiac and skeletal muscle proteins. Adherent PEC (2 × 10^5 cells per well) were used as APC to present proteins to CDL 7.8.1. After a 2-hr incubation at 37°C, the PEC were washed, and 1 × 10^5 CDL 7.8.1 cells were added for 24 hr. IL-2 secretion in the supernatants was assessed by proliferation of the IL-2-dependent line CTLL. Incorporation of [*methyl*-^{3}H]thymidine by the CTLL cells was measured; results are mean ± SD values for quadruplicates. Proteins were tested at a final concentration of 300, 100, and 30 μg/ml, and results were similar; only data from the 100 μg/ml concentration are shown. PEC or CDL 7.8.1 alone incorporated <500 cpm of [^{3}H]thymidine.

ment of PEC and antibodies to I-E^d inhibited the response of CDL 7.8.1 cells to cardiac myosin, showing that processing of myosin is required for reactivity and that the recognized epitope is restricted by the I-E^d molecule (data not shown). To address the possibility that a lack of response was due to processing difficulties with the native forms of these muscle proteins, CNBr digests of all of the above-tested proteins were also prepared. CDL 7.8.1 cells showed reactivity only to the digests of mouse and rat cardiac myosin (data not shown); these data indicate that CDL 7.8.1 is specific for an epitope of rodent cardiac myosin. Thus, we generated a T-cell hybridoma from an autologous T cell that was specific for its own cardiac myosin and which could be used to detect myosin being processed within the heart.

Isolation of Residential Cardiac APC. To examine whether functional APC existed within the normal myocardium, we first isolated a nonmyocyte cell population from the myocardium by serial collagenase and trypsin digestions and discontinuous Percoll gradients. The isolated cells contain an adherent cell population and had an approximate density between 1.04 and 1.06 g/ml on discontinuous Percoll gradients. FACS analysis (Fig. 2 *Left*) revealed that 24% are CD45$^+$ and 16% are class II MHC$^+$. All the class II MHC$^+$ cells are also CD45$^+$, suggesting that functional antigen presentation occurs by bone-marrow-derived, class II MHC$^+$ cells and does not occur on cardiac myocytes, fibroblasts, or endothelial cells. This result may be similar to the murine equivalent of the class II MHC$^+$/CD45$^+$ dendritic cell in the rat heart, identified by Spencer and Fabre (34). Approximately 15% of the isolated cells from normal hearts are CD11b$^+$ (data not shown), suggesting that a residential macrophage population normally exists within the mouse myocardium.

Detection of Endogenously Processed Cardiac Myosin on Cardiac APC. We hypothesized that cardiac myosin was endogenously processed and presented on these residential APC within the myocardium. To test this, we isolated cardiac APC from normal mice in the presence of chloroquine to inhibit any potential processing of myosin during the isolation procedure and added these cells to the cardiac myosin-specific T-cell hybridoma CDL 7.8.1. We found that myosin–class II MHC complexes were present on cardiac APC isolated from normal mouse hearts (Fig. 3), but were not present on spleen cells taken from the same mice. In addition, PEC did not stimulate CDL 7.8.1 in the absence of added antigen (see Fig. 5). Therefore, the processing of cardiac myosin occurs only within the myocardium. We also examined several other H-2^d mouse strains for the presence of myosin–class II MHC complexes. Normal cardiac APC isolated from C.B-17, SCID, B10.D2, and DBA/2J mice stimulate CDL 7.8.1, showing that endogenous processing of

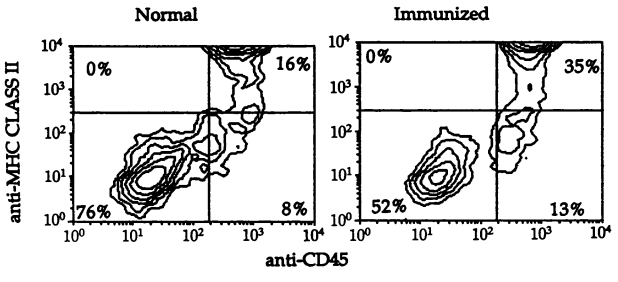

FIG. 2. Flow cytometric analysis of normal cardiac APC (*Left*) and cardiac APC from myosin-immunized H-2^d mice (*Right*). Two-color labeling was done by using biotinylated-MKD6 (anti-I-A^d) and fluorescein isothiocyanate-conjugated I3/2.3 (anti-CD45). Results were similar from cells isolated on two separate occasions. The quadrants were assigned based on unstained APC and APC singly stained with either MKD6 or I3/2.3.

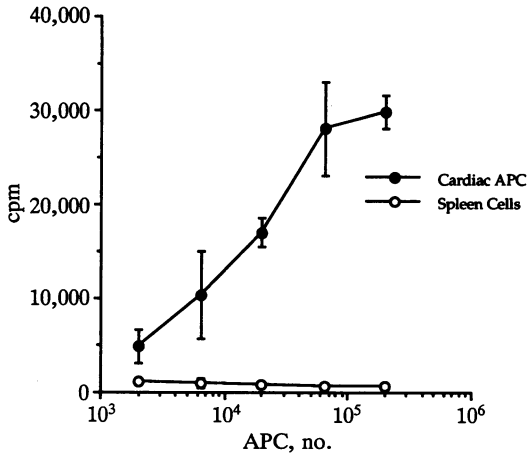

FIG. 3. Detection of endogenous myosin–class II MHC complexes on cardiac APC from normal BALB/cByJ mice. Cardiac APC were isolated in the presence of 100 μM chloroquine, as described in the text, and incubated with the cardiac myosin-specific T-cell hybridoma CDL 7.8.1 without the addition of exogenous antigen. Results were similar with cardiac APC isolated from five separate experiments. Stimulation of the hybridoma was determined as described for Fig. 1, except that cardiac APC were used. Results are mean ± SD values for quadruplicates.

myosin by residential cardiac APC is a phenomenon found in a variety of mouse strains (data not shown). Cardiac APC stimulated an unrelated hybridoma (21DO34.4) but only stimulated it when its specific antigen (ovalbumin) was added to the culture. These studies support our hypothesis that cardiac myosin is endogenously processed and presented by residential cardiac APC.

Effect of Autoimmune Myocarditis on Endogenous Processing and Presentation of Cardiac Myosin. We were next interested to learn whether induction of autoimmune myocarditis affected the endogenous processing and presentation of cardiac myosin within the myocardium. To evaluate this further, BALB/cByJ mice were immunized either with mouse cardiac myosin, as described, or with CFA alone and the cardiac APC isolated either 14 or 21 days later. We have previously shown that histologic myocarditis is first evident between days 12 and 16 after immunization and is most severe on day 21 (35). On day 14, cardiac APC from the CFA only and myosin-immunized groups stimulated hybridoma CDL 7.8.1 slightly more than the normal controls (Fig. 4). However, by day 21, cardiac APC from the myosin-immunized mice stimulated significantly more than the other groups, and this finding was consistent when the APC were compared on a per cell basis. Thus, cardiac APC from hearts with active autoimmune myocarditis present endogenous myosin complexes better. We also performed FACS analysis of cardiac APC isolated 21 days after myosin immunization and found almost double the number of CD45$^+$/class II MHC$^+$ cells (Fig. 2 *Right*). These data directly demonstrate that stimulation of the immune system by initiation of a cardiac-specific autoimmune response increases both the expression of myosin–class II MHC complexes on myocardial APC and the number of class II MHC$^+$ cells in the target organ and enhances the functional capacity of these APC.

Myosin–class II MHC complexes were not generated during the isolation procedure, as shown by the following experiments. We first confirmed that cardiac myosin required processing and that this processing was inhibited by chloroquine. We prepared PEC as APC because they do not constitutively express myosin peptide–class II MHC complexes, and treated these PEC during the adherence step with chloroquine (100 μM), a lysosomotropic amine that has been well-studied and shown to inhibit antigen processing (36).

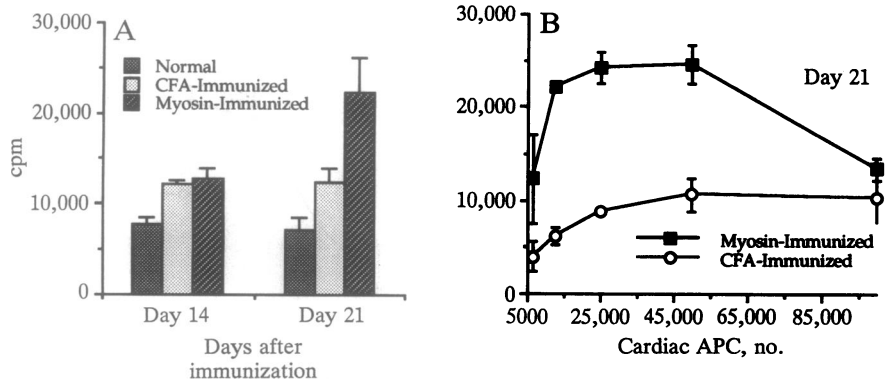

FIG. 4. Detection of myosin–class II MHC complexes on cardiac APC isolated 14 or 21 days after immunization by the cardiac myosin-specific T-cell hybridoma CDL 7.8.1. BALB/cByJ mice were immunized with either cardiac myosin or CFA as described in text, and the cardiac APC were isolated in the presence of 100 μM chloroquine either 14 or 21 days later and added as shown to 1×10^5 CDL 7.8.1 cells; IL-2 production was determined as described for Fig. 1. (*A*) Cardiac APC at 2×10^4 cells per well. (*B*) Titration of the same APC used in *A* from either CFA only or myosin-immunized mice. Results were similar with APC isolated from two separate experiments.

After chloroquine treatment, the PEC were washed, and three different sources of exogenous cardiac myosin were added for a 2-hr incubation at 37°C; subsequently the cells were washed and fixed with 1% paraformaldehyde to eliminate presentation of native, but not peptide, antigen (30, 37). The response of hybridoma CDL 7.8.1 to myosin presented on these PEC is shown in Fig. 5. Chloroquine completely inhibited processing and presentation of both native myosin and myosin released from a cell-free extract of the digested hearts. Note that presentation of CNBr myosin peptides was preserved. As expected, no endogenous cardiac myosin complexes were detected on these PEC. Second, we isolated cardiac APC with 100 μM chloroquine, according to the standard protocol, and then fixed the cells with 1% paraformaldehyde. These chloroquine-treated, fixed cardiac APC stimulated CDL 7.8.1 cells without added antigen (data not shown). Based on these experiments we believe that chloroquine inhibits processing of myosin released from myocytes during the isolation procedure, thereby eliminating the possibility that myosin–class II MHC complexes were formed *in vitro*.

DISCUSSION

Although implicit in the experimental results of several autoimmune diseases (27, 38, 39), this report directly demonstrates that the inducing self-protein in an autoimmune disease is endogenously processed and presented within the target organ, before any form of tissue injury. We show that using a cardiac myosin-specific T-cell hybridoma, CDL 7.8.1, we could detect myosin–class II MHC complexes in a class II $MHC^+/CD45^+$ cell population isolated directly from the hearts of normal mice and that induction of autoimmune

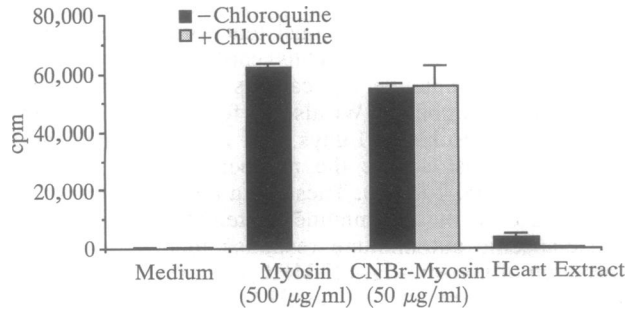

FIG. 5. Effect of chloroquine on ability of PEC to process and present protein to hybridoma CDL 7.8.1. PEC were obtained, as described in text, and 2×10^5 cells per well were treated with 100 μM chloroquine during the 2-hr adherence step. After being washed, 1×10^5 CDL 7.8.1 cells were added to the PEC together with myosin, CNBr myosin, medium only, or a cell-free extract from collagenase- and trypsin-digested mouse hearts, as described in the text. The heart extract contained total protein at 1.5 mg/ml. After 24 hr, supernatants were removed, and IL-2 production was determined, as described for Fig. 1. Results are mean ± SD values for quadruplicates.

myocarditis increased the expression of myosin–class II MHC complexes on cardiac APC.

The significance of these studies is that expression of myosin–class II MHC complexes on cardiac APC does not require myocardial tissue injury. Therefore, there must be local turnover of cardiac myosin or degradative intermediates that are captured by the residential APC. By capturing polypeptides from local myosin turnover, the residential APC may protect the host by preventing myosin exposure to the systemic immune system or, at least, by keeping exposure to a subthreshold level. Self-reactive T cells are then not exposed to myosin because lymphocytes are not present in the normal heart. Thus, the residential APC may have an important role in protective homeostasis in health. In the setting of tissue injury, the APC would be important in maintaining this protection, not only because more myosin is available to the immune system, but because tissue injury recruits lymphocytes into the heart. This hypothesis is supported by the fact that autoimmune sequelae are a rare complication of myocardial infarction (40).

The mechanisms controlling the autoimmune response are still unknown. Our studies demonstrate, however, that self-antigen–class II MHC complexes exist before tissue injury—therefore, availability of self-antigen is not the limiting step. Schwartz (41) has suggested that costimulatory signals from the APC are critical for determining whether self-reactive T cells will become stimulated upon engagement of self-antigen–class II MHC. According to this theory, if myosin-reactive T cells contact myosin–class II MHC on cardiac APC, tolerance could be maintained due to lack of the required costimulatory signals by the cardiac APC. Immunization with myosin could start the sequence of events that allow the cardiac APC to provide appropriate costimulation to myosin-reactive T cells *in vivo* to initiate autoimmune myocarditis. Another possibility is that the number of myosin–class II MHC complexes on cardiac APC is below the threshold required to stimulate a myosin-reactive T cell and that myosin immunization increases the number of these complexes. We previously reported that myosin-stimulated T cells could adoptively transfer autoimmune myocarditis into SCID mice, but this transfer would occur only if the SCID mice were immunized with myosin (27). Activation of the myosin-stimulated T cells by Con A allowed the T cells to successfully transfer myocarditis without myosin immunization. Thus, the activation state of the T cell may also be critical for induction of the autoimmunity.

The expression of myosin–class II MHC complexes *in vivo* is enhanced during the process of autoimmune myocarditis, as shown by increased reactivity of hybridoma CDL 7.8.1 to cardiac APC isolated 21 days after immunization, a time point when histologic inflammation is most severe. The intriguing possibility that ongoing tissue injury releases self-antigen and thereby contributes to the pathogenesis of autoimmunity, unfortunately, cannot be resolved from these experiments.

The facilitation of complex expression as tissue injury progresses could result from several factors. Increased complexes could be detected as class II MHC-bearing cells are recruited into the myocardium during the inflammatory process and endocytose myosin from dying myocytes. Although the number of APC isolated from inflamed hearts is increased, it is never more than double the number isolated from normal hearts. This result is consistent with the FACS analysis (Fig. 2 *Right*) that showed almost double the number of class II MHC$^+$ cells from day 21 inflamed hearts. The release of cytokines such as interferon γ from activated T cells could increase class II MHC expression and activate both residential cardiac APC and infiltrating macrophages, thereby enhancing the efficiency of antigen presentation. It is also possible that increased detection of myosin–class II MHC complexes resulted from release of myosin from the immunization sites. However, we feel this is unlikely for two reasons. (*i*) If APC that had processed myosin at the immunization site migrated to the heart, they would be expected to circulate through the spleen at some point. However, complexes were never detected in splenocytes of myosin-immunized mice on day 14 or 21 after immunization (unpublished observation). (*ii*) Complex expression is not increased on day 7 (unpublished observation), even though myosin is present at the immunization site. Therefore, increased expression of myosin complexes appeared to correlate with histologic progression of disease severity.

Isolation of a functional APC population from the myocardium is an important finding from these studies. Our studies reconfirm the previous findings of Hart and Fabre (42), Spencer and Fabre (34), and Steiniger *et al.* (43) that both class II MHC$^+$/CD45$^+$ and class II MHC$^-$/CD45$^+$ interstitial cell populations are found within the normal rodent myocardium and expand these observations by directly showing that these cells are functional APC. In addition to the probable role of these cardiac APC in initiating autoimmune myocarditis, they may also play a role in cardiac transplant rejection. Studies by Larsen *et al.* (44) have shown that a class II MHC$^+$/CD45$^+$ dendritic cell population migrates from the donor heart into the recipient spleen, suggesting another pathway for sensitization of the host immune system against donor MHC antigens. A similar route could exist in the initiation of autoimmune myocarditis, where cardiac APC migrate to the spleen of myosin-immunized mice in sufficient numbers to participate in activating myosin-specific T cells. Herskowitz *et al.* (45) and Rose *et al.* (46) have shown that normal human cardiac myocytes do not express class II MHC molecules, although the data of Rose *et al.* suggest that endothelial cells in normal human hearts do express class II MHC molecules. The functional APC in these studies are not endothelial cells or myocytes because they lack CD45.

Our studies show that myosin–class II MHC complexes are expressed *in vivo* on cardiac APC before the induction of autoimmune myocarditis and, thus, tissue injury is not required to release self-antigen. In addition, expression of myosin–class II MHC complexes on cardiac APC is facilitated during the disease process. These local APC could have an important role in protection against exposure of self-protein to the systemic immune system.

We thank Drs. Brian Evavold, David Hagerty, and Robert Graziano for critical reading of the manuscript and Erin Geringer for technical assistance. S.C.S. is a Howard Hughes Medical Institute Postdoctoral Research Fellow. P.M.A. is an Established Investigator of the American Heart Association. This work was supported by National Institutes of Health Grant AI-31238.

1. Kappler, J. W., Staerz, U., White, J. & Marrack, P. C. (1988) *Nature (London)* **332,** 35–40.
2. Kappler, J. W., Noehm, N. & Marrack, P. (1987) *Cell* **49,** 273–280.
3. Hengartner, H., Odermatt, B., Schneider, R., Schreyer, M., Walle, G., MacDonald, H. R. & Zinkernagel, R. M. (1988) *Nature (London)* **336,** 388–390.
4. Bill, J., Kanagawa, O., Woodland, D. L. & Palmer, E. (1989) *J. Exp. Med.* **169,** 1405–1419.
5. Kisielow, P., Blüthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. (1988) *Nature (London)* **333,** 742–746.
6. Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russell, J. H. & Loh, D. Y. (1988) *Nature (London)* **336,** 73–79.
7. Murphy, K. M., Heimberger, A. B. & Loh, D. Y. (1990) *Science* **250,** 1720–1723.
8. Burkly, L. C., Lo, D. & Flavell, R. A. (1990) *Science* **248,** 1364–1368.
9. Murphy, K. M., Weaver, C. T., Elish, M., Allen, P. M. & Loh, D. Y. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 10034–10038.
10. Schönrich, G., Kalinke, U., Momburg, F., Malissen, M., Schmitt-Verhulst, A.-M., Malissen, B., Hämmerling, G. J. & Arnold, B. (1991) *Cell* **65,** 293–304.
11. Lorenz, R. G. & Allen, P. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5220–5223.
12. Weiss, S. & Bogen, B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 282–286.
13. Stockinger, B. & Hausmann, B. (1988) *Eur. J. Immunol.* **18,** 249–253.
14. Winchester, G., Sunshine, G. H., Nardi, N. & Mitchison, N. A. (1984) *Immunogenetics* **19,** 487–491.
15. Bikoff, E. K., Yu, H. & Eckhardt, L. A. (1988) *Eur. J. Immunol.* **18,** 341–348.
16. Wekerle, H., Schwab, M., Linington, C. & Meyermann, R. (1986) *Eur. J. Immunol.* **16,** 1551–1557.
17. Falk, K., Rötzschke, O., Stevanvic, S., Jung, G. & Rammensee, H.-G. (1991) *Nature (London)* **351,** 290–296.
18. Jardetzky, T. S., Lane, W. S., Robinson, R. A., Madden, D. R. & Wiley, D. C. (1991) *Nature (London)* **353,** 326–329.
19. Rudensky, A. Y., Rath, S., Preston-Hurlburt, P., Murphy, D. B. & Janeway, C. A., Jr. (1991) *Nature (London)* **353,** 660–662.
20. Rudensky, A. Y., Preston-Hurlburt, P., Hong, S.-C., Barlow, A. & Janeway, C. A., Jr. (1991) *Nature (London)* **353,** 622–627.
21. Hunt, D. F., Henderson, R. A., Shabanowitz, J., Sakaguchi, K., Michel, H., Sevilir, N., Cox, A. L., Appella, E. & Engelhard, V. H. (1992) *Science* **255,** 1261–1263.
22. Zamvil, S. S. & Steinman, L. (1990) *Annu. Rev. Immunol.* **8,** 579–621.
23. Singh, V. K., Yamaki, K., Donoso, L. A. & Shinohara, T. (1989) *J. Immunol.* **142,** 1512–1517.
24. Bouvet, J.-P., Couderc, J., Bouthillier, Y., Franc, B., Decreusefond, C. & Mouton, D. (1989) *J. Immunol.* **143,** 1537–1542.
25. Witebsy, E. R., Rose, N. R., Kerplan, K., Paine, J. R. & Egan, R. W. (1957) *J. Am. Med. Assoc.* **164,** 1439–1447.
26. Neu, N., Rose, N. R., Beisel, K. W., Herskowitz, A., Gurri-Glass, G. & Craig, S. W. (1987) *J. Immunol.* **139,** 3630–3636.
27. Smith, S. C. & Allen, P. M. (1991) *J. Immunol.* **147,** 2141–2147.
28. Shiverick, K. T., Thomas, L. L. & Alpert, N. R. (1975) *Biochim. Biophys. Acta* **393,** 124–133.
29. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256,** 495–497.
30. Allen, P. M. & Unanue, E. R. (1984) *J. Immunol.* **132,** 1077–1079.
31. Lorenz, R. G., Blum, J. S. & Allen, P. M. (1990) *J. Immunol.* **144,** 1600–1606.
32. Yamada, K. A. & Corr, P. B. (1992) *J. Cardiovasc. Electrophysiol.,* **3,** 209–224.
33. Trowbridge, I. S. (1978) *J. Exp. Med.* **148,** 313–323.
34. Spencer, S. C. & Fabre, J. W. (1990) *J. Exp. Med.* **171,** 1841–1851.
35. Smith, S. C. & Allen, P. M. (1992) *Circ. Res.* **70,** 856–863.
36. Ziegler, K. & Unanue, E. R. (1982) *Proc. Natl. Acad. Sci. USA* **78,** 175–178.
37. Shimonkevitz, R., Kappler, J. W., Marrack, P. & Grey, H. M. (1983) *J. Exp. Med.* **158,** 303–316.
38. Zamvill, S., Nelson, P., Trotter, J., Mitchell, D., Knobler, R., Fritz, R. & Steinman, L. (1985) *Nature (London)* **317,** 355–358.
39. Haskins, K., Portas, M., Bergman, B., Lafferty, K. & Bradley, B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8000–8004.
40. Braunwald, E. (1988) *Heart Disease* (Saunders, Philadelphia), pp. 1521–1523.
41. Schwartz, R. H. (1990) *Science* **248,** 1349–1356.
42. Hart, D. N. J. & Fabre, J. W. (1981) *J. Exp. Med.* **153,** 347–361.
43. Steiniger, B., Klempnauer, J. & Wonigeit, K. (1984) *Transplantation* **38,** 169–175.
44. Larsen, C. P., Morris, P. J. & Austyn, J. M. (1990) *J. Exp. Med.* **171,** 307–314.
45. Herskowitz, A., Ahmed-Ansari, A., Neumann, D., Beschorner, W., Rose, N., Soule, L., Burek, C., Sell, K. & Baughman, K. (1990) *J. Am. Coll. Cardiol.* **15,** 624–632.
46. Rose, M. L., Page, C., Hengstenberg, C. & Yacoub, M. H. (1990) *Hum. Immunol.* **28,** 179–185.