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Heart-specific autoantibodies can be eluted from the hearts of Coxsackievirus B3-infected mice

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SUMMARY

This study was undertaken to determine if immunoglobulin G (IgG) antibodies could be eluted from the hearts of mice with Coxsackievirus B3-induced autoimmune myocarditis and to characterize the immunoreactivity of any elutable autoantibodies. Susceptible (A/J) and resistant (B10.A) mice were administered the virus or the control treatment and killed at various times after treatment. Acid eluates from pooled heart tissue from each treatment group and each time were tested for IgG reactivity with normal heart tissue by immunohistochemistry and with normal heart extracts by Western immunostaining. Eluates from infected A/J mice reacted strongly with syngeneic heart and modestly with syngeneic skeletal muscle tissue. Eluates from infected B10.A or control mice of either strain exhibited little reactivity with either tissue. Tissue reactivity was similar when allogeneic tissue was used as the substrate. Eluates from infected A/J mice recognized the heavy chain of cardiac myosin and several other cardiac antigens by Western immunostaining while eluates from the other treatment groups exhibited little or no reactivity with any normal heart constituents. These results indicate that *in vivo* IgG deposition occurs in the hearts of mice with post-infectious autoimmune myocarditis and that the specificity of these antibodies is similar to that reported for serum from animals with this disease. The mechanism(s) leading to myocardial IgG deposition and its possible role in pathogenesis remain to be elucidated.

Keywords autoimmunity myocarditis Coxsackievirus B3 heart eluate

INTRODUCTION

Autoimmune disorders are typically characterized by circulating antibodies to self antigens (Davis, Percy & Russell, 1977; Hawkins, Cheah & Dawkins, 1980; Morimoto *et al.*, 1982; Granholm *et al.*, 1985). Through the use of sensitive immunoassays autoantibodies are commonly detected in individuals with no clinical indications of ongoing pathology (Guilbert, Dighiero & Avrameas, 1982; Mach *et al.*, 1984; Stefansson *et al.*, 1985). Autoantibodies unrelated to pathogenesis may contribute to homeostasis by binding to senescent molecules, perhaps marking them for elimination (Daar & Fabre, 1981; Kay *et al.*, 1982; Grabar, 1983). Thus, distinguishing between benign autoantibodies and those that may be relevant to, or associated with, pathogenesis is critical to understanding autoimmune disease.

In organ-specific autoimmune disease a key test is to demonstrate that autoantibodies bind to the organ *in situ* (Male, Roitt & Hay, 1980; Jasin, 1985; Schwartz & Datta, 1989). Such a demonstration would suggest that the antibodies recognize and specifically bind to self antigen(s) while eluting antibody from the target organ would facilitate characterization of the antigenic specificity of the most relevant autoantibodies, i.e. those that localize *in vivo*.

In the murine model of autoimmune myocarditis following Coxsackievirus B3 (CB3) infection, affected animals develop autoantibodies that specifically recognize heart tissue (Wolfgram *et al.*, 1986). Susceptibility to autoimmune myocarditis is genetically restricted mainly to mice from inbred strains possessing the A background genes, e.g. A/J, while strains derived from the C57BL lineage, e.g. B10.A, are resistant to post-CB3 autoimmune myocarditis (Wolfgram *et al.*, 1986; Rose *et al.*, 1986). Serum from susceptible animals reacts principally with the heavy chain of cardiac myosin (Alvarez *et al.*, 1987) and is mediated by IgG antibodies (Wolfgram, Beisel & Rose, 1985; Alvarez *et al.*, 1987) which do not cross-react with CB3 (Neu *et al.*, 1987b). Unpublished observations from this laboratory indicate that there is substantial IgG deposition in the hearts of susceptible animals with post-CB3 autoimmune myocarditis.

This study was initiated to determine if antibody could be eluted from the hearts of susceptible and resistant mice infected with CB3 and to test the eluates for immunoreactivity with both

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tissue and extracts from normal hearts. Acid elution of hearts from infected susceptible, but not control or resistant, animals yielded IgG antibodies reactive with normal heart tissue and with the heavy chain of cardiac myosin.

MATERIALS AND METHODS

Mice

A/J and B10.A mice from colonies maintained at the Animal Resources Center, The Johns Hopkins Oncology Center, were used for all experiments.

Treatment

Approximately 50 mice from each strain, 2–3 weeks old, were infected with CB3, propagated in Vero monkey kidney cells by the procedure of Wolfgram *et al.* (1986). Animals were infected intraperitoneally by a single 0·1 ml inoculum of a 10^5 tissue culture infective dose of CB3 diluted 1:3 in RPMI 1640 (Sigma Chemical Co., St Louis, MO). Infected mice were housed in micro-isolator cages with lactating syngeneic females and were provided with sterile food and acidified water *ad libitum*. Equal numbers of treatment control mice from each strain were given an identical inoculation of a similarly diluted uninfected Vero cell lysate (VCL).

Sampling

Blood was collected from eight to 12 animals from each strain and each treatment group on days 9, 15, 21 and 28 after treatment. Each animal was anaesthetized and bled retro-orbitally. Since the eluates (see below) were prepared from pools of hearts from identically treated animals, serum pools were prepared for each strain and treatment group at each sampling time. Thus if an eluate was obtained from ten animals, equivalent volume aliquots of serum from those animals were used to form the corresponding pool. All sera were stored at −70°C until tested.

The heart of each mouse was excised by severing the atrioventricular septum. A 2–3 mm thick section cut from the mid-ventricular region perpendicular to the long axis of the heart was fixed in 10% buffered formalin for haematoxylin and eosin staining. The remainder of each heart was used for immunoglobulin elution.

Immunoglobulin elution

Hearts from animals in each group were pooled and minced in 40 ml of RPMI 1640 under aseptic conditions. The minced tissue was transferred to sterile tubes and centrifuged for 10 min at 850 ***g*** at room temperature (RT). After three additional washes in RPMI, the tissue was resuspended in 11 ml of RPMI containing 160 U/ml of collagenase (Sigma Chemical Co.). The suspension was incubated at 37°C for 1 h with constant rocking and decanted into a Petri plate where the residual tissue was ground between the frosted ends of sterile microscope slides. This preparation was centrifuged at RT for 5 min at 50 ***g***.

The digested tissue was washed with RPMI containing 1 mM phenylmethyl sulphonylfluoride (PMSF) and twice with RPMI alone. The pellet was resuspended in 20 ml of 0·02 M citric acid (Pankewycz, Migliorini & Madaiio, 1987) in phosphate-buffered saline (PBS), pH 3·2, and incubated for 3 h at 37°C with constant rocking. Insoluble material was pelleted by centrifugation at RT for 10 min at 1200 ***g***. The supernatant was neutralized in 20 ml of 1 M Tris-base and dialysed in the cold against several changes of PBS containing 0·02% sodium azide (PBS/A). The protein concentration of the dialysed eluates was determined by the bicinchoninic acid (Pierce Chemical Co., Rockford, IL) procedure (Smith *et al.*, 1985). Dialysed eluates were tested for the presence of heart-reactive antibodies.

Demonstration that the elution procedure selects for tissue-bound antibody

Seven CB3-infected and ten VCL-inoculated mice were killed 24 days after treatment and processed as described above. The volume of each supernatant was determined and each was treated with sufficient PMSF and sodium azide to yield concentrations of 1 mM and 0·02%, respectively. The IgG concentration of each was determined by capture ELISA using vinyl 96-well plates (Costar, Cambridge, MA) coated overnight at 4°C with 6 μg/ml of affinity purified goat anti-mouse IgG antibody (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) in carbonate/bicarbonate buffer. The wells were washed five times with PBS, pH 7·4, containing 0·05% Tween 20 (Sigma Chemical Co.), blocked for 1 h at RT with PBS containing 0·1% gelatin (PBS/G), and washed as above. Purified mouse IgG (Sigma Chemical Co.) serially diluted from 1000 to 7 ng/ml in PBS/G was dispensed in triplicate. Each supernatant was tested in triplicate neat and diluted 1:3, 1:6, or 1:12 with RPMI. Each eluate was tested in triplicate neat and diluted 1:3 in PBS/A. After 2 h at RT, the wells were washed as above and incubated for 2 h at RT with horseradish peroxidase conjugated goat anti-mouse IgG antibody (Kirkegaard & Perry Laboratories, Inc.) diluted 1:6000 in PBS/G. The wells were washed and incubated for 2 h at RT with the substrate, 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (Kirkegaard & Perry Laboratories, Inc.) and the optical density of each well at 410 nm was determined.

Optical density values for wells containing known amounts of mouse IgG were used to calculate a linear standard curve from which the IgG concentration of each sample was estimated. The data were normalized by expressing the results in ng of IgG per heart.

Immunohistochemical staining of normal heart tissue

Heart and skeletal muscle from untreated, age-matched syngeneic mice were used to test the heart eluates for tissue-reactive antibodies. Normal mice from each strain were killed and perfused with saline. The heart and right quadriceps femoris muscle were excised, flash-frozen in OCT compound (Miles Inc., Elkhart, IN) and cut into 5-μm sections which were mounted on microscope slides and fixed with acetone.

Tissue sections were rinsed twice with Tris-buffered saline (TBS), pH 7·6 (0·05 M Tris-base, 0·85% NaCl) and placed in a humid chamber at RT. Endogenous peroxidase activity was depleted by incubating the tissue with TBS containing 0·5% Carnation non-fat dry milk (Carnation Co., Los Angeles, CA), 0·5% hydrogen peroxide, and 1% normal goat serum for 30 min. The sections were rinsed in TBS containing 0·5% dry milk (TBS/M) for 5 min and placed in TBS/M containing 1% normal goat serum (TBS/MG) for 10 min. After 5 min of washing with TBS/M, the eluates (neat) were placed on both syngeneic and allogeneic tissue for 1 h before washing with TBS/M for 5 min. A polyclonal, biotinylated goat $F(ab')_2$ anti-mouse IgG $F(ab')_2$-

specific reagent (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:1000 in TBS/MG was placed on the sections for 30 min. Sections were rinsed for 5 min each in TBS and in TBS/M and incubated with an avidin/biotinylated horseradish peroxidase complex (Vector Laboratories, Burlingame, CA) for 1 h. After washing as above the sections were incubated with 0·06% 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0·003% hydrogen peroxide in 0·05 M Tris buffer, pH 7·6, for 10 min. The sections were washed for 5 min in TBS and then in running tap water for 5 min before being immersed in 0·5% copper sulphate in 0·15 M NaCl for 1 min to enhance colour contrast. The sections were washed for 2 min under running tap water, counter-stained in Mayers haematoxylin, and washed for 5 min under running tap water before being dehydrated and covered.

When each assay was performed, additional sections were processed as reagent controls to assure the reliability of the assay; some samples were retested to assess reproducibility. Serum samples of proven heart-reactivity as well as normal serum, both diluted 1:100, were used as positive and negative controls, respectively.

Sections were examined by light microscopy and the heart and skeletal muscle evaluated for each of four staining patterns: fibrillary, linear, sarcolemmal, and interstitial (Wolfgram *et al.*, 1985; Neumann *et al.* 1990). Each pattern on each tissue was assigned a semi-quantitative score reflecting the extent and intensity of staining relative to that observed with serum from untreated syngeneic mice. Scoring was as follows: 0 = no detectable staining; 1 = weak focal staining; 2 = moderate focal staining; 3 = moderate staining of the entire myocardium; and 4 = intense staining of the entire myocardium. For each eluate the four scores for each tissue were summed to yield a total immunoreactivity score.

Western immunostaining of normal heart extract

Hearts from age-matched, untreated syngeneic mice were excised, minced, and weighed. A 10% (w/v) extract was prepared by homogenizing the tissue in 125 mM Tris-HCl, 1% sodium dodecyl sulphate (SDS), and 0·05% dithiothreitol, pH 6·8 (Giometti *et al.* 1980). The homogenate was poured through an extraction buffer wetted gauze to remove debris and stored at −130°C until used.

The extract, diluted 1:10 in SDS-polyacrylamide gel electrophoresis buffer, was heated at 100°C for 5 min and 80 μl loaded into a 68 mm long well of a 3–16% linear polyacrylamide minigel prepared in 1 M Tris-base, 1 M bicine, pH 8·6. Four microlitres of prestained molecular weight standards (BRL, Bethesda, MD) were loaded into a reference well. Electrophoresis was performed at RT at 55 milliamps until the tracking dye reached the bottom of the gel (~1·5 h). Each gel was placed on a polyvinylidine difluoride membrane (ISS-Enprotech, Hyde Park, MA); the top and bottom of each gel and the position of each standard was marked by perforating the membrane. Heart proteins were electrotransferred to the membrane in 25 mM Tris-base, 192 mM glycine at 4°C (0·2 amps, 1 h). Membranes were dried in the dark at RT and similarly stored for up to 2 weeks before use.

For each membrane, the distance from the origin to each molecular weight marker and to the bottom of the gel was measured. The acrylamide concentration at the position of each marker was estimated by linear interpolation (Andrews, 1986). A molecular weight standard curve based on the log acrylamide concentration and on the log molecular weight of each marker was determined for each membrane and used to estimate the molecular weight of the antigens detected on the corresponding membrane.

Each membrane was cut into 2–3 mm wide strips which were wetted with 100% methanol, dipped into distilled water, and immersed in troughs containing 2 ml of 38 mM Tris-HCl, 7 mM Trizma-base, 0·15 M NaCl, 0·05% Tween 20, and 3% (w/v) Carnation non-fat dry milk (TBS/Blotto), pH 7·4, for 1 h at RT with constant rocking. Subsequent incubations were performed similarly using a constant volume of 2 ml/strip. The strips were washed for 5 min with 43 mM Tris-HCl, 11 mM Trizma-base, 15 mM NaCl, and 0·05% Tween 20 (TBS/T), pH 7·4. Individual strips were incubated with each eluate (neat) or pooled serum (diluted 1:200) from each treatment group overnight at 4°C. The strips were washed three times with TBS/T for 10 min each and incubated with a biotinylated goat $F(ab')_2$ anti-mouse IgG Fc region-specific antibody (Jackson ImmunoResearch Laboratories) diluted 1:2000 in TBS/T for 1 h at RT. The strips were washed as before and incubated for 1 h with an Extravidin/biotinylated horseradish peroxidase complex (Sigma Chemical Co.) diluted 1:40000 in TBS/T. After washing as above the strips were incubated with 2 mM DAB in 100 mM Tris-buffer containing 1·7 mM NaCl and 0·01% hydrogen peroxide until optimal colour development occurred on the positive control strip. The strips were washed twice in distilled water and dried overnight in the dark at RT.

Each time the assay was performed, both positive and negative (normal) serum controls were tested as were a full set of reagent controls. Assays in which any of the controls failed to perform as expected were repeated. The number of bands of immunoreactivity on each strip was recorded and the position of each recorded, in order to estimate the relative molecular weights of the reactive antigens.

RESULTS

Demonstration that the elution procedure selects for tissue-bound antibody

Mincing and washing the hearts four times in relatively large volumes of buffer reduced the soluble IgG concentration by 94–98% (Fig. 1). Collagenase digestion of the washed tissue released additional IgG amounting to 25–29% of that found in the first wash. Supernatants from the third post-collagenase washes yielded estimates of 14 ng of IgG/heart for infected mice and 7 ng/heart for control mice. Citric acid elution resulted in a 12- to 40-fold increase in recoverable IgG.

Immunohistochemical staining of normal syngeneic heart tissue

Heart eluates from CB3-infected A/J mice exhibited IgG reactivity with all four of the structural elements examined in both heart and skeletal muscle. IgG-defined linear, fibrillary, and interstitial patterns of reactivity in heart tissue were greater than in skeletal muscle tissue. Eluates from A/J control mice exhibited little or no reactivity with either heart or skeletal muscle. The total immunoreactivity score (Fig. 2a) indicated that heart eluates from infected mice possessed greater reactivity with heart than with skeletal muscle while eluates from control mice possessed negligible tissue immunoreactivity. Tissue reac-

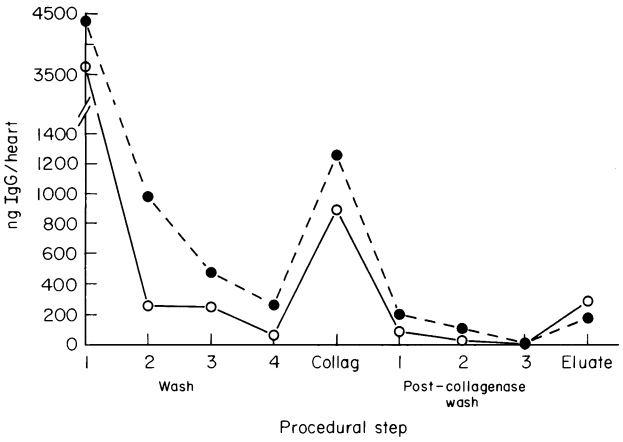

Fig. 1. IgG concentration (ng/heart) in supernatants from the preparative steps preceding citric acid elution of antibody from the hearts of mice infected with CB3 (●) or administered the uninfected Vero cell lysate (VCL) control treatment (○). The procedure consisted of four successive washes of minced heart tissue with RPMI 1640, collagenase digestion, and three successive washes with RPMI 1640 prior to acid elution (eluate).

tivity was evident as early as 9 days after infection and persisted through the end of the experiment, day 28 after infection.

Eluates from both CB3-infected and control B10.A mice exhibited weak IgG reactivity with each of the four structural features; scores for the individual patterns were similar. Staining by eluates from hearts of infected B10.A mice was similar to staining obtained with eluates from the hearts of control mice. Total immunoreactivity scores (Fig. 2b) suggest a transient increase in tissue reactivity 15 days after treatment.

When tissue reactivity was evaluated using allogeneic (B10.A) heart and skeletal muscle, the eluates from A/J mice generally exhibited similar scores as were observed for syngeneic tissue (Fig. 2c) although there was an apparent increased recognition of interstitial antigens from allogeneic skeletal muscle. Eluates from control A/J mice exhibited little or no reactivity with allogeneic heart or skeletal muscle (Fig. 2c). Heart eluates from infected and control B10.A mice were somewhat more reactive with interstitial antigens from allogeneic (A/J) heart and skeletal muscle than with syngeneic interstitial antigens (Fig. 2d).

Western immunostaining of normal heart extract

Pooled serum from CB3-infected susceptible A/J mice reacted

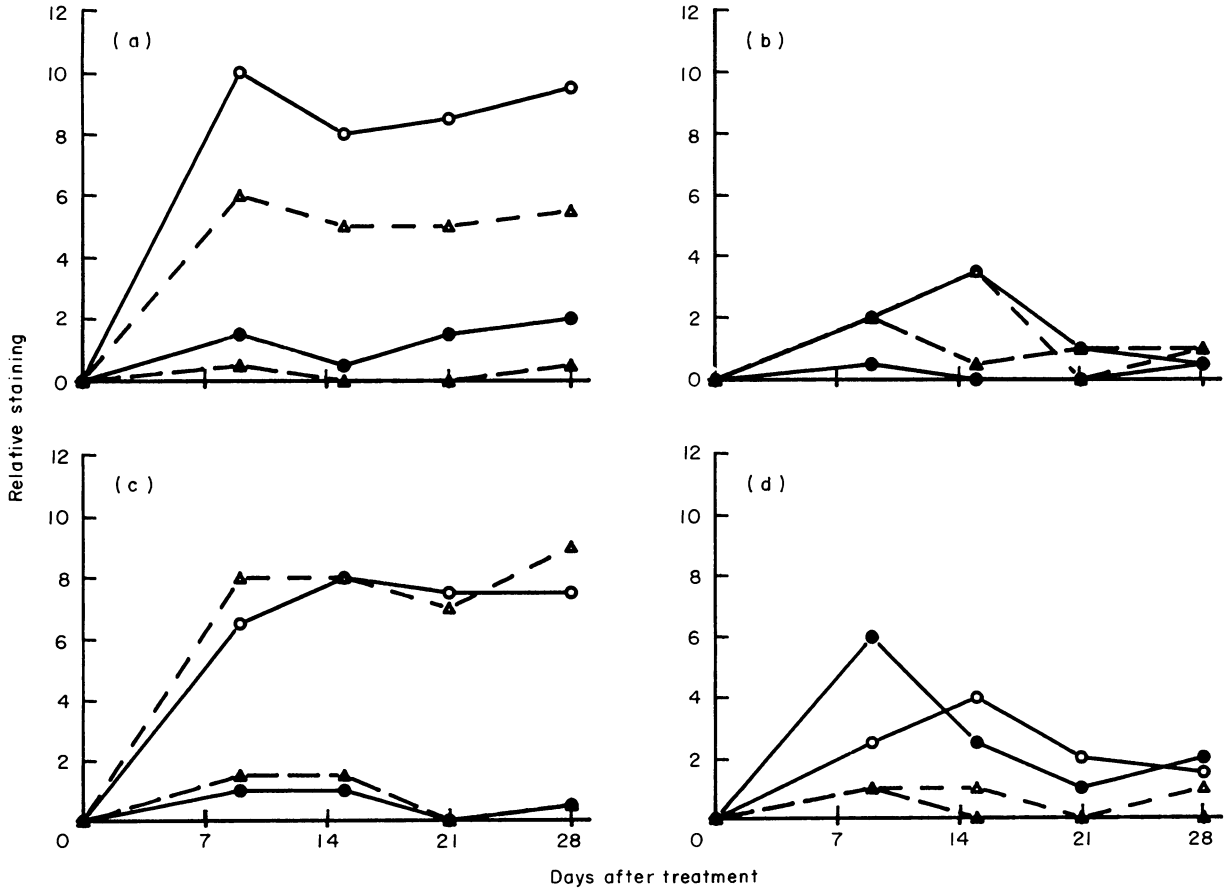

Fig. 2. Relative staining of normal heart and skeletal muscle tissue by IgG in heart eluates from mice infected with CB3 or administered the uninfected Vero cell lysate (VCL) control treatment. (a) Heart eluates from infected and control susceptible A/J mice tested against syngeneic heart and skeletal muscle. (b) Heart eluates from infected and control resistant B10.A mice tested against syngeneic heart and skeletal muscle. (c) Heart eluates from infected and control susceptible A/J mice tested against allogeneic (B10.A) heart and skeletal muscle. (d) Heart eluates from infected and control resistant B10.A mice tested against allogeneic (A/J) heart and skeletal muscle. O, Heart, post-CB3; ●, heart post-VCL; △, skeletal muscle, post-CB3; ▲, skeletal muscle, post-VCL.

Table 1. Molecular weights of cardiac antigens recognized by pooled serum and heart eluates from susceptible A/J and resistant B10.A mice infected with CB3 or administered the uninfected Vero cell lysate (VCL) control treatment

	A/J		B10.A	
Day	Serum	Eluate	Serum	Eluate
CB3-infected				
9	ND	37	160 225	None
15	231	None	160 225	None
21	116 126 157 165 181	37 180 200	160 225	None
28	116 126 157 165 181	37 180 200	160 225	None
Treatment control				
9	242	180	160 225	None
15	231	None	160 225	None
21	242	None	160 225	None
28	None	None	160 225	None

Animals were killed 9, 15, 21 and 28 days after treatment. The estimated molecular weights of the reactive antigens are expressed in kilodaltons and were determined by Western immunostaining.

ND, no protein detected.

with as many as five cardiac antigens by western immunostaining (Table 1). The number of reactive antigens increased over the course of the experiment; reactivity was principally directed against antigens between 116 and 181 kD. Serum from treatment control A/J mice exhibited reactivity with single antigens of 231 or 242 kD depending upon the time of sampling (Table 1).

Antibody activity eluted from the hearts of mice 21 days after treatment is illustrated in Fig. 3. The eluate prepared from A/J mice 9 days after infection reacted with a 37-kD antigen while those prepared 21 or 28 days after infection recognized this antigen and antigens of 180 and 200 kD (Table 1). The eluate from treatment control mice on day 9 reacted with a heart antigen of 180 kD; no other reactivity was observed with any of the other eluates from treatment control mice (Table 1).

Pooled serum from both CB3-infected and VCL-treated resistant B10.A mice consistently reacted with cardiac antigens of 160 and 225 kD (Table 1). Heart eluates from both the CB3-infected and the control B10.A mice did not react with any cardiac antigens (Fig. 3).

DISCUSSION

This study demonstrates that heart-reactive IgG antibodies can be eluted from the hearts of susceptible strain, A/J, mice with post-CB3 autoimmune myocarditis. Little or no heart-reactive IgG antibody could be eluted from the hearts of treatment control A/J mice or from CB3-infected or control resistant strain, B10.A, mice. These results suggest that the eluted antibodies may contribute to the continuing pathogenesis associated with autoimmune myocarditis.

Antibody eluted from the hearts of CB3-infected A/J mice reacted with each of four microanatomical features found in normal heart tissue. Total immunoreactivity scores suggest that the eluates contain antibodies with greater specificity or affinity for cardiac antigens than for skeletal muscle antigens. Alternatively, differences in reactivity between heart and skeletal muscle tissues might be due to quantitative differences in antigen expression in the two tissues or to differences in the amount of antibody eluted from the hearts of the variously treated animals. Table 2 indicates that immunoreactivity is not correlated with the total amount of protein present in the eluate, thus differences in antibody specificity and/or affinity are likely to account for the immunostaining results.

Wolfgram *et al.* (1985) found that circulating heart-reactive IgG arising subsequent to CB3 infection included both heart-specific and heart cross-reactive antibodies. By Western immunostaining this reactivity was largely directed against the heavy chain of myosin and consisted of IgG antibodies specific for the cardiac isoform of myosin as well as those which recognized epitopes shared by cardiac and skeletal muscle myosin (Alvarez *et al.*, 1987). When mice susceptible to autoimmune myocarditis were immunized with cardiac myosin, antibodies to both the cardiac and skeletal muscle isoforms were elicited (Neu *et al.*, 1987a). Unpublished data from this laboratory indicate that circulating IgG antibodies elicited in A/J and in B10.A mice by immunization with mouse cardiac myosin recognize different antigenic fragments of myosin.

Western immunostaining indicated that antibodies to the heavy chain of cardiac myosin were eluted from the hearts of CB3-infected susceptible A/J mice. This polypeptide has a molecular weight of ~ 180–220 kD in the electrophoresis system described here (Neumann *et al.*, 1990). Control A/J mice and both CB3-infected and control autoimmune myocarditis-resistant B10.A mice do not develop myocarditis and do not have heart-bound IgG. The relative absence of heart reactivity by these eluates suggests that myocardial injury may be a prerequisite for the induction of heart autoantibodies. Thus tissue damage, rather than polyclonal B cell activation (Swartzwelder *et al.*, 1988), may be the key to the induction of the heart-reactive antibodies. This may result from the increased accessibility of cardiac antigens due to myocyte necrosis or to the non-specific increase in antigen presenting cells associated with local myocardial inflammation.

Heart eluates from both strains exhibited similar reactivity against both syngeneic and allogeneic tissue suggesting that at this relatively gross level there are no substantial differences in the antigenic composition of muscle tissue from either strain. Thus, while susceptibility to post-CB3 autoimmune myocarditis may be regulated by genes independent of the major histocompatibility complex (Wolfgram *et al.*, 1986; Rose *et al.*, 1986; Beisel & Traystman, 1988), susceptibility does not appear to be

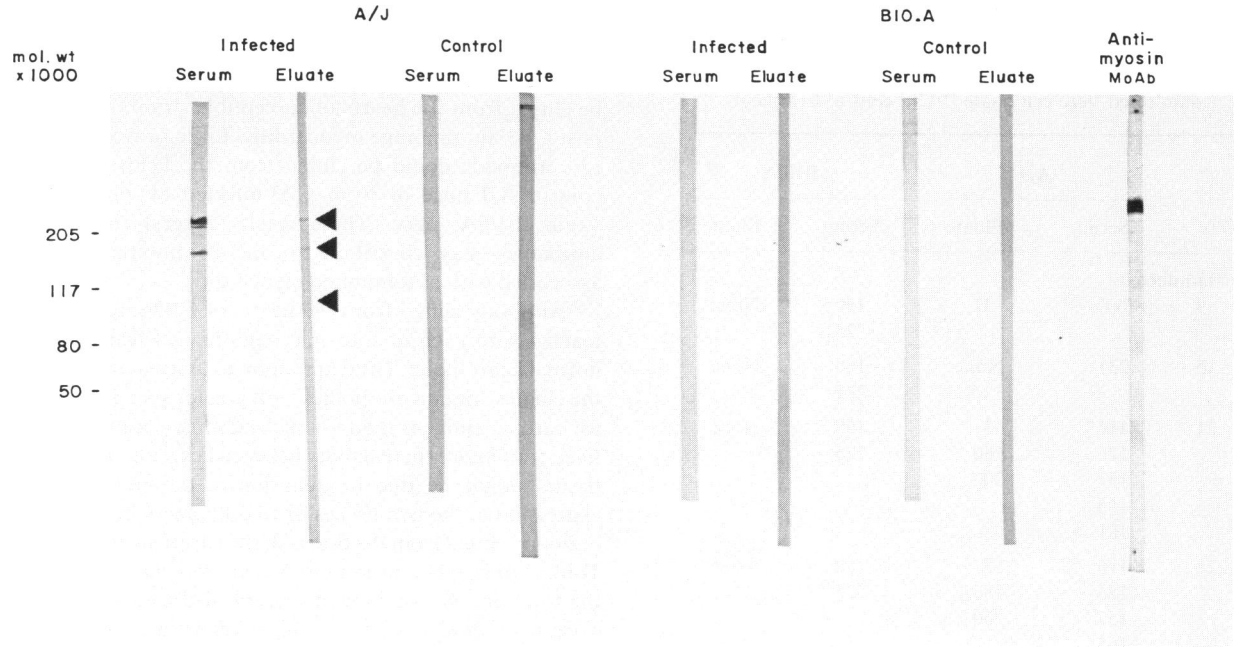

Fig. 3. Western immunostaining of syngeneic whole heart extracts by pooled serum and heart eluates from susceptible A/J and resistant B10.A mice infected with CB3 or administered the uninfected Vero cell lysate (VCL) control treatment. The serum and eluates were obtained 21 days after treatment. For reference, the reactivity of an anti-myosin monoclonal antibody (Pan Myosin RPN.1169: Amersham Corp., Arlington Heights, IL) with the extract is included.

Table 2. Total protein content and immunoreactivity of eluates from hearts of CB3-infected and control (VCL-inoculated) A/J and B10.A mice

		Eluates from A/J (susceptible) mice			Eluates from B10.A (resistant) mice		
			Immunoreactivity with heart			Immunoreactivity with heart	
	Days post-treatment	Protein (μg/heart)	Tissue	Extract	Protein (μg/heart)	Tissue	Extract
CB3-infected	9	203	++++	+	1370	0	0
	15	1655	+++	0	357	0	0
	21	860	+++	++	230	0	0
	28	143	++++	++	663	0	0
VCL-inoculated (control)	9	630	+	+	473	+	0
	15	ND	+	0	790	+	0
	21	ND	+	0	597	+	0
	28	ND	+	0	898	+	0

Protein content expressed as μg/heart. Immunoreactivity (IgG) with normal syngeneic heart tissue was defined on the basis of the combined immunohistochemical staining score: + (0–3), ++ (4–6), +++ (7–9), and ++++ (10–12). Immunoreactivity (IgG) with extracts of normal syngeneic hearts by Western immunostaining was defined as follows: 0 = no staining, + = weak staining, and ++ = strong staining.

ND, no protein detected.

associated with differences in the normal tissue distribution of antigens between susceptible and resistant strain animals. If the induction of anti-heart antibodies in A/J mice was due to the presence of a heart antigen unique to that strain then one would not expect antibody from A/J mice to be equally reactive with B10.A heart tissue. However, this does not preclude the possibility that the expression of the relevant antigen(s) may be altered in one or both strains during virus infection.

The processes contributing to myocardial IgG deposition remain to be determined. Western immunostaining indicates that only some of the heart-reactive specificities found in the serum are bound to the tissue *in vivo*. While this may reflect absorption of specific serum antibodies, direct immunohistochemistry indicates that heart infiltrates include surface membrane IgG^+ cells (data not shown) which may secrete antibody locally within the injured tissue. *In vivo* IgG deposition implies

an F(ab')$_2$-mediated mechanism, although Fc-mediated deposition may be important, especially in inflammatory diseases where Fc receptor-rich infiltrating cells may promote antibody deposition. However, extensive infiltration does not appear to be a prerequisite for myocardial IgG deposition since IgG is also observed in hearts which are only mildly inflamed and may occur in areas well removed from inflammatory lesions.

These results raise the question of whether IgG antibodies contribute to the ongoing pathogenesis of autoimmune myocarditis. Such immunopathogenicity might best be demonstrated by transferring the disease to normal, syngeneic recipients with autoimmune serum or purified IgG. While many investigators consider it unlikely that antibody alone is responsible for pathogenesis, thyroiditis (Vladutiu & Rose, 1971), collagen arthritis (Stuart *et al.*, 1982), and pemphigus (Anhalt *et al.*, 1982; Roscoe *et al.*, 1985) have been successfully transferred by humoral factors. In some cases antibody appears not to be directly cytotoxic, but rather promotes antibody dependent cell-mediated cytotoxic or other hypersensitivity reactions (Calder, McLeman & Irvine, 1973; Jaroszewski, Sundick & Rose, 1978). In many experimental models of autoimmune disease, pathogenicity has been associated with T effector cells (Trentham, Dynesius & David, 1978; Taurog, Sandberg & Mahowald, 1983; Williams *et al.*, 1987; Mahi-Brown, Yule & Tung, 1987). Since cell-mediated immunity may contribute to the pathogenesis of autoimmune myocarditis (Huber & Lodge, 1986; Huber, Heintz & Tracy, 1988), both serum and cell transfer experiments are currently being performed in this laboratory.

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