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Immune Response to Laminin, a Noncollagenous Glycoprotein of Basement Membrane, in a Syngeneic Murine System[1] (41484)

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Abstract. The immune response to the noncollagenous, attachment glycoprotein of basement membrane (laminin) was studied in a syngeneic murine system. Delayed-type hypersensitivity (DTH) was assayed in C57BL/6 mice by measuring footpad swelling following challenge with connective tissue antigens. Mice receiving a single sensitizing injection of laminin in Freund's complete adjuvant (FCA) developed a significant DTH response which peaked on Day 7, 24 hr after challenge with laminin or collagenase-treated laminin. Laminin-sensitized mice failed to show any significant footpad swelling when challenged with types I or IV collagen, or fibronectin throughout these experiments. Normal mice displayed no significant DTH when challenged with these collagenous or noncollagenous connective tissue antigens. Adoptive transfer of laminin-sensitized spleen cells into normal mice resulted in significant DTH responsiveness to challenge with laminin; depletion of T cells from the immune spleens abrogated this response. Twenty-four hours after challenge with laminin, histology of the footpad lesions of laminin-sensitized mice revealed a mononuclear cell infiltrate, characteristic of a DTH response. Mice receiving repeated injections of laminin in Freund's incomplete adjuvant (FIA) developed significant antibody responses as detected by the enzyme-linked immunosorbent assay (ELISA). Furthermore Pronase, but not collagenase, treatment of laminin destroyed its antigenicity. Laminin immune sera showed no reactivity when assayed on fibronectin or collagen types I–V. No cross-reactivity was exhibited by murine anti-type IV or anti-type I collagen antisera with laminin. These studies demonstrate the ability of isologous laminin to induce antigen-specific cell-mediated and humoral immunity in a murine model.

Basement membranes are extracellular tissue structures which separate parenchymal cells from underlying connective tissue matrices. Basement membranes provide a supportive framework for epithelial (1, 2) and endothelial cells, and function as a barrier to the passage of macromolecules (3). Several basement membrane-specific proteins have been identified: type IV collagen (3), laminin (4), or GP-2 (5, 6), a heparan sulfate proteoglycan (7, 8) and entactin (9, 10).

Tissues containing a high concentration of basement membrane (i.e., lung and kidney) are frequent sites of immunologically mediated injury; however, until recently, specific immunity to basement membrane components has been primarily examined through the use of specific antibodies to aid in the characterization of its biochemical

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[3] Abbreviations used: ABTS, 2,2′-azino-di-[3-ethylbenzthiazoline-6-sulfonate]; anti-Thy 1.2, monoclonal anti-mouse Thy 1.2 antibody; C, guinea pig complement; DTH, delayed-type hypersensitivity; EHS, Engelbroth–Holm/Swarm sarcoma; ELISA, enzyme-linked immunosorbent assay; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; PBS–Tween–BSA, PBS (pH 7.8) containing 0.05% Tween 20 and 1% bovine serum albumin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; T cell, thymus-derived lymphocyte.

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properties and tissue distribution. We have previously demonstrated the ability of types IV and I collagen to induce cell-mediated and humoral immunity in a murine model (11–13). Antibodies to type IV collagen have been identified in the sera of patients with systemic sclerosis (scleroderma) (14). The levels of these anti-collagen autoantibodies correlated directly with the extent of interstitial lung disease in this patient population. These studies implicate type IV collagen as an autoantigen and immunity to this basement membrane protein may be involved in initiating and/or perpetuating this disease process.

Laminin, a disulfide-linked glycoprotein is present in all basement membranes in quantities approximately equal to type IV collagen (4). Although the role of laminin in the basement membrane has not been completely determined, it appears to function in cell attachment similar to fibronectin with type I collagen; however, laminin is biochemically and immunologically distinct from fibronectin (4, 15). Sakashita *et al.* (16) demonstrated that laminin specifically binds to heparin and heparan sulfate. They suggest that an interaction of laminin with heparan sulfate proteoglycans of the basement membrane may contribute to the structural integrity of basement membranes *in vivo*. In addition, Ekblom *et al.* (17) have suggested that laminin may be involved in the increased cell adhesiveness observed during the aggregation phase of kidney morphogenesis. In the present study, we have examined the ability of laminin, isolated from the Engelbroth–Holm/Swarm (EHS) sarcoma, to induce an immune response in a syngeneic murine system. Our data demonstrate cell-mediated immunity to laminin, following a single sensitizing exposure, and the development of a strong antibody response after repeated immunizations with this basement membrane antigen.

Materials and Methods. *Animals.* C57BL/6 female mice (Laboratory Animal Medicine, Medical University of South Carolina) were used at 6–8 weeks of age.

Antigens. Laminin was isolated from the EHS sarcoma as described by Timpl *et al.* (4). Tumors were homogenized in 3.4 *M* NaCl (0.05 *M* Tris–HCl, pH 7.4) at 4° to remove soluble extracellular proteins. The residue was extracted twice with 0.5 *M* NaCl (0.05 *M* Tris–HCl, pH 7.4) at 4°. All extractions contained the protease inhibitors phenylmethane sulfonyl fluoride (160 μg/ml; Sigma Chemical Co., St. Louis, Mo.) and *N*-ethylmaleimide (1.25 mg/ml; Sigma). Type IV collagen was removed from the 0.5 *M* NaCl extract by increasing the salt concentration to 1.7 *M* and centrifuging the solution at 10,000 rpm. The supernatant fluid containing laminin was dialyzed against 2 *M* urea (0.05 *M* Tris–HCl, pH 8.6) and passed over a diethylaminoethyl (DEAE) cellulose column equilibrated with the same buffer. The unbound material containing laminin was concentrated by ultrafiltration (Diaflo filter XM 100, Amicon Corp., Lexington, Mass.). The concentrated sample was chromatographed on an agarose A-1.5m column, equilibrated and eluted with 1 *M* $CaCl_2$ (0.05 *M* Tris–HCl, pH 7.4). Laminin appeared in the void volume and was lyophilized following dialysis against 0.05% acetic acid.

Collagen types I and IV were prepared as described previously (11, 18). Type I collagen was isolated from murine tail tendon. The EHS sarcoma served as the source of type IV collagen. Following pepsinization of the homogenized tumor tissue, the collagen was purified by sequential acetic acid solubilizations, precipitation with NaCl, and DEAE-cellulose chromatography. Human fibronectin was obtained from Collaborative Research Inc. (Waltham, Mass.).

Analysis of antigens. Laminin and collagen types I and IV were assayed for purity by amino acid analysis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and susceptibility to protease-free bacterial collagenase. No evidence of other collagen types or noncollagenous impurities were observed in the collagen preparations as previously described (11). Following reduction with 2-mercaptoethanol, the laminin preparation exhibited two homogeneous bands on SDS–polyacrylamide gels (4%) with mo-

lecular weights slightly greater than 200,000 and 400,000. Treatment of laminin with protease-free bacterial collagenase prior to analysis by SDS–PAGE did not alter the gel patterns. Treatment of types I and IV collagens in the same manner eliminates their gel bands (11). Thus the laminin preparation appears free of collagenous contaminants.

Collagenase and Pronase treatments. Laminin was dissolved in 0.005 *M* $CaCl_2$ (0.05 *M* Tris–HCl, pH 7.6) at a concentration of 2.5 mg/ml. Bacterial collagenase (*Clostridium histolyticum;* Millipore Corp., Freehold, N.J.) was purified by the method of Peterkofsky and Diegelmann (19) and was free of noncollagenase proteolytic activity as we have previously demonstrated (11). Purified bacterial collagenase or Pronase (Calbiochem–Behring Corp., La Jolla, Calif.) was added at concentrations of 20 units/125 μl and 0.2 mg/125 μl, respectively. The samples were incubated at 37° for 18 hr, and subsequently dialyzed extensively against phosphate-buffered saline (PBS).

Delayed-type hypersensitivity. Five micrograms laminin in 0.1 ml of 0.1 *M* acetic acid was emulsified with an equal volume of Freund's complete adjuvant (FCA; Gibco, Grand Island, N.Y.) and injected subcutaneously (sc) in the abdomen; control mice were untreated. DTH was measured by footpad swelling in response to antigenic challenge as described previously (11). Briefly, mice were injected intradermally in the plantar surface of the hindfoot with 5 μg antigen in a volume of 0.03 ml. Footpad thickness was determined 4 and 24 hr post-challenge with a micrometer and compared to measurements observed prior to antigen injection. The data are expressed as the mean percentage footpad swelling ± standard error (SE):

$$\% \text{ footpad swelling} = \frac{\text{mm after challenge} - \text{mm before challenge}}{\text{mm before challenge}} \times 100.$$

Passive transfer. Spleens were removed from normal or laminin-sensitized mice 7 days after immunization. The sensitized spleen cells were left untreated or were incubated with monoclonal murine anti-Thy 1.2 antibody (Anti-Thy 1.2; New England Nuclear, Boston, Mass.) plus guinea pig complement (C; M.A. Bioproducts, Walkersville, Md.) as previously described (11). Twenty-five million untreated or T-cell-depleted laminin-sensitized spleen cells, or 25 × 10^6 normal spleen cells were injected intraperitoneally (ip) in 0.5 ml PBS into normal mice. Two days after cell transfer, recipient mice were challenged in the footpad with 5 μg laminin and footpad swelling was assayed 24 hr later.

Histology. Normal and laminin-sensitized mice were challenged in the footpad on Day 6 with 5 μg laminin. Twenty-four hours later, mice were sacrificed and their feet fixed in a neutral gluteraldehyde solution, followed by embedding in glycol methacrylate (Polysciences, Inc., Warrington, Pa.). Sections were stained with hematoxylin and eosin, and examined by light microscopy (11).

Preparation of antisera. Rabbit antibodies against types I and IV collagen were prepared as described previously (14). Rabbit anti-laminin serum was obtained using the same immunization protocol. A New Zealand white rabbit was injected in each hind footpad on Days 0 and 14 with 0.5 mg laminin in FCA. On Days 23 and 33, the animal was injected sc with 1 mg laminin in Freund's incomplete adjuvant (FIA; Gibco). Blood was collected from the ear on Days 30 and 40, and by cardiac puncture on Day 41. A gamma globulin fraction was obtained following ammonium sulfate precipitation (14).

Mice were immunized on Days 0, 14, 21, 28, and 35 by sc injections of 50 μg laminin, type IV collagen, or type I collagen dissolved in 0.1 *M* acetic acid and emulsified with an equal volume of FIA. Immune and normal mice were bled from the ophthalmic venous plexus on Day 42. The sera from each group (at least four mice per group) were pooled and stored at −70°.

Enzyme-linked immunosorbent assay (ELISA). Antibodies to laminin were detected using the ELISA as we have de-

scribed previously (13, 14). Serial dilutions of rabbit antibodies (1 mg/ml) and immune mouse sera in PBS (pH 7.8) containing 0.05% Tween 20 and 1% bovine serum albumin (PBS–Tween–BSA), were applied to microtiter wells (Flow Laboratories, McLean, Va.) coated with 1.25 μg laminin and incubated for 45 min. After washing, 100 μl of peroxidase-conjugated goat anti-rabbit immunoglobulin (IgG, M, A) antibodies (1:500, Cappel Laboratories, Cochranville, Pa.) or peroxidase-conjugated goat anti-mouse immunoglobulin (IgG, M, A) antibodies (1:250; Cappel) in PBS–Tween–BSA were added, the plates incubated for an additional 45 min, and then washed. One hundred microliters of the substrate, 0.03% ABTS (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate], Sigma), was added to each well in 0.1 *M* citrate buffer (pH 4.0) with 0.05% H_2O_2, and after incubation for 1 hr, the absorbance was read at 414 nm on a Titertek Multiskan (Flow). All assays were performed in duplicate and the results are expressed as the mean absorbance value.

Absorption assays. Repeated absorptions of normal and immune mouse sera were performed to remove anti-laminin antibody activity. A 1:20 dilution of each serum was plated as described above. After a 45-min incubation, the sera were transferred to other laminin-coated wells. This process was continued for up to four absorptions, and the assay was completed by the addition of peroxidase-conjugated immunoglobulin and substrate. In order to control for the loss of volume resulting in reduction of the sera activity on laminin following each transfer, mouse anti-type I and anti-type IV collagen sera were plated on laminin. These sera were then transferred to

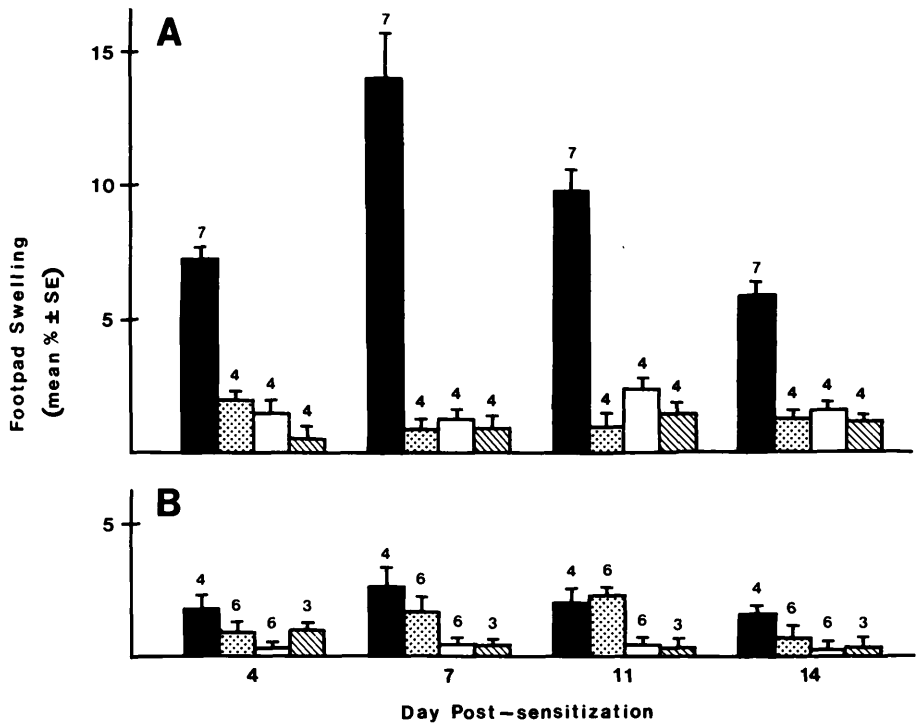

FIG. 1. DTH response of (A) mice sensitized with 5 μg laminin in FCA and (B) normal mice challenged on Days 3, 6, 10, and 13 postsensitization with laminin (■), type I collagen (▦), type IV collagen (□), and fibronectin (▨). DTH was assayed 24 hr postchallenge and the results are expressed as mean percentage footpad swelling ± SE. The number of mice assayed is indicated above each bar.

plates coated with their respective antigen. No significant loss of reactivity with the collagen antigens was observed by the anti-collagen sera after the four absorptions with laminin.

Results. The ability of C57BL/6 mice to mount a DTH response to homologous laminin is shown in Fig. 1. Groups of mice were immunized with 5 μg laminin in FCA on Day 0; normal controls were untreated. Mice were challenged with 5 μg laminin, type I collagen, type IV collagen, or fibronectin on Days 3, 6, 10, and 13 postsensitization. Footpad swelling was assayed 4 and 24 hr later, with maximal swelling occurring at 24 hr. No significant swelling was observed at 4 hr, indicating that the response was not due to an antibody-mediated Arthus reaction or to an immediate hypersensitivity response. As shown in Fig. 1A, mice sensitized and challenged with laminin demonstrated significant DTH on Day 4 (7.2% ± 0.5), with the response peaking 7 days postsensitization (13.8% ± 1.9). Laminin-sensitized mice showed no significant DTH when challenged with types I or IV collagen, or fibronectin. Normal control mice (Fig. 1B) failed to show significant swelling at any time when challenged with laminin, types I or IV collagen, or fibronectin.

To eliminate the possibility that a collagenous protein contaminant in our laminin preparation was contributing to the observed DTH response, mice were challenged with collagenase and Pronase-treated laminin (Table I). Normal and laminin-sensitized mice showed no significant footpad swelling on Day 7, 24 hr after challenge with Pronase-treated laminin (Table I, Expt 1). Challenge of these same mice with untreated laminin immediately following assay of the footpad swelling on Day 7 resulted in significant footpad swelling ($P < 0.025$) on Day 8 in the laminin-sensitized mice only. Laminin-sensitized mice challenged on Day 6 with collagenase-treated laminin displayed significant DTH 24 hr later; normal controls showed no significant swelling (Table I, Expt 2). These data indicate that noncollagenous protein antigens are responsible for the

TABLE I. COLLAGENASE AND PRONASE TREATMENT OF LAMININ USED FOR DTH CHALLENGE[a]

Experiment	Sensitization	No. of mice	Challenge 1	Footpad swelling (mean % ± SE) Day 7	Challenge 2	Footpad swelling (mean % ± SE) Day 8
1	None	3	Pronase-treated laminin	0.16 ± 0.13	Laminin	0.3 ± 0.30
	Laminin	4	Pronase-treated laminin	0.92 ± 0.46	Laminin	7.3 ± 0.50
2	None	3	Collagenase-treated laminin	0.94 ± 0.31		
	Laminin	4	Collagenase-treated laminin	10.4 ± 1.10		

[a] Mice were sensitized with 5 μg laminin in FCA; control mice were untreated. Mice were challenged on Day 6 with 5 μg Pronase or collagenase-treated laminin preparations. Footpad swelling was determined at 24 hr (Day 7). Mice in Experiment 1 were immediately challenged with untreated laminin following assay of footpad swelling on Day 7. Footpad swelling was determined in these groups after 24 hr (Day 8).

observed DTH response in the laminin-sensitized mice.

The ability of laminin-sensitized spleen cells to adoptively transfer DTH responsiveness to normal syngeneic mice is shown in Table II. Spleen cells were obtained from laminin-sensitized mice on Day 7, the peak of the DTH response (Fig. 1). Twenty-five million sensitized spleen cells were injected ip into normal syngeneic mice, which were challenged 48 hr later with laminin; these mice displayed significant footpad swelling 24 hr after challenge. Mice receiving an ip injection of 25×10^6 sensitized spleen cells which were pretreated with a monoclonal murine anti-Thy 1.2 antibody plus C failed to display significant footpad swelling when challenged with laminin. Injection of 25×10^6 normal C57BL/6 spleen cells into normal mice did not transfer reactivity to laminin. These data indicate that Thy 1.2-positive T lymphocytes are responsible for mediating the DTH response to laminin.

Histological examination of the footpad lesions of laminin-sensitized mice on Day 7, 24 hr after challenge with laminin, revealed an inflammatory response with a predominantly mononuclear cell infiltrate (Figs. 2A and B). Histologic sections obtained from the footpads of normal mice after challenge with laminin showed no significant inflammation (Figs. 2C and D). The identification of a mononuclear cell infiltrate in the footpad lesions of the laminin-immune mice is a feature characteristic of a DTH response.

The ability of C57BL/6 mice to elicit an antibody response to homologous laminin was examined using the ELISA. Rabbit antisera against laminin were included in all assays as positive controls and to determine reproducibility of the procedure. Rabbit antisera to types I or IV collagen display no significant reactivity when assayed on laminin-coated wells. No antibody was detected in the sera of C57BL/6 mice displaying DTH as a result of a single injection of laminin; however, sera obtained from mice receiving repeated sc injections of 50 μg laminin in FIA displayed significant antibody reactivity (laminin titers >320) when assayed on laminin (Fig. 3). Minimal background absorbance was exhibited by normal mouse sera at all dilutions and represent background values (Fig. 3). Sera obtained from mice sensitized to type I and type IV collagen using the same immunization schedule as with laminin displayed no significant reactivity when assayed on laminin (Fig. 3); however, these sera do exhibit high antibody responses when assayed on wells coated with their respective immunogens (13). The anti-laminin mouse sera did not react with wells coated with fibronectin or types I–V collagen (data not shown).

Treatment of the laminin-coated wells with purified bacterial collagenase (11) had no effect on the ability of the anti-laminin sera to react with laminin (Fig. 4A); Pronase treatment of laminin-coated wells eliminated the observed anti-laminin reactivity of the immune sera. Repeated absorptions of the anti-laminin sera on laminin-coated wells were performed to determine if all laminin-specific activity could be removed, since a single absorption did not remove all activity (data not shown). As shown in Fig. 4B, reactivity of laminin immune sera was reduced to background by sequential absorptions on laminin-coated wells. Thus, the antibody reactivities of these immune sera are spe-

TABLE II. ADOPTIVE TRANSFER OF DTH TO LAMININ USING UNTREATED AND T-CELL-DEPLETED SENSITIZED SPLEEN CELLS

Sensitization[a]	Cell treatment	Challenge	No. of mice	Footpad swelling (mean % ± SE)
None	None	Laminin	4	1.5 ± 0.38
Laminin	None	Laminin	6	9.7 ± 1.20
Laminin	Anti-Thy 1.2 + C	Laminin	8	1.3 ± 0.36

[a] Donor mice were either untreated or immunized with 5 μg laminin in FCA.

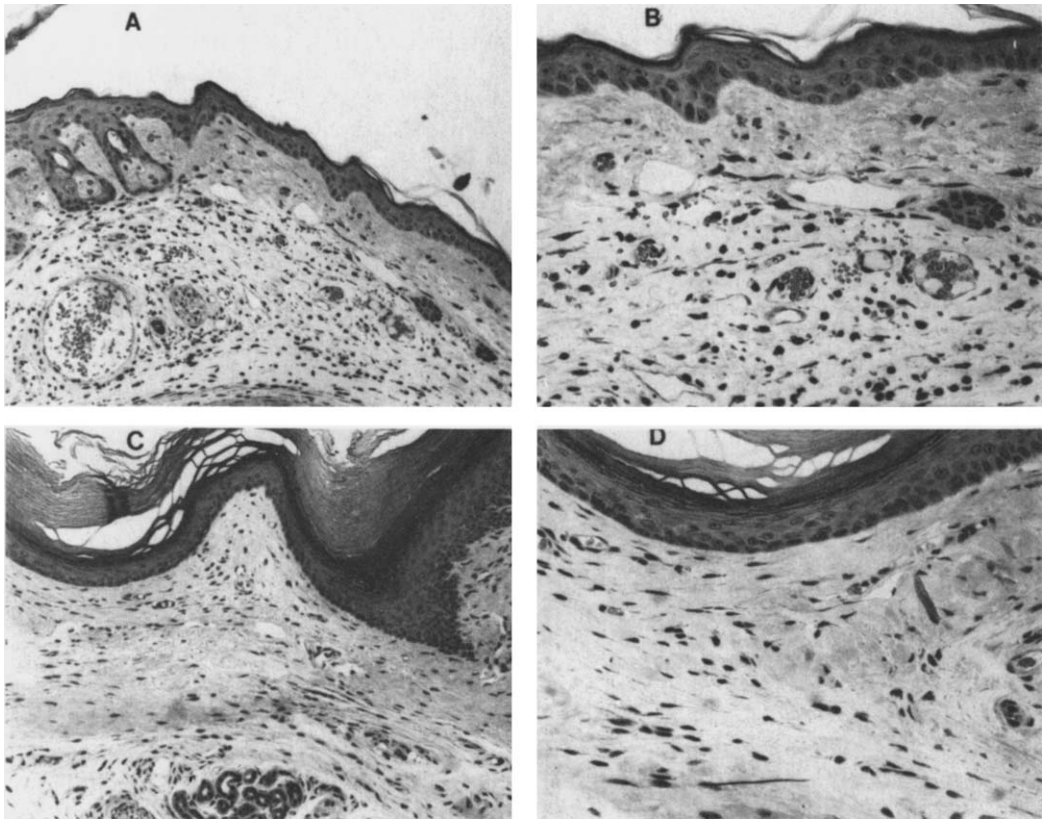

FIG. 2. Histological examination of the footpad lesions from laminin-sensitized and normal mice 7 days postsensitization and 24 hr after challenge with laminin. Sections from a laminin-sensitized mouse showing (A) an inflammatory response (×145) and (B) the presence of a mononuclear cell infiltrate (×285). Sections from a normal mouse challenged with laminin showing no inflammation (C, ×145; D, ×285).

cific for a noncollagenous protein of the basement membrane.

Discussion. Noncollagenous macromolecules are integral components of basement membranes interacting with type IV collagen to generate this extracellular matrix. Laminin, the major noncollagenous glycoprotein of basement membrane, has been shown by indirect immunofluorescence to be associated with a wide variety of mammalian tissues (4, 15) and, by electron microscopy, to be localized primarily in the lamina rara of the basement membrane (15, 20). Laminin, consisting of two polypeptide chains (220,000 and 440,000 daltons) linked by disulfide bonds, is biochemically and antigenically distinct from type IV collagen and fibronectin (4). The Engelbroth–Holm/Swarm (EHS) sarcoma is a transplantable murine tumor which produces an extracellular matrix of basement membrane components (21). The present studies were performed using the EHS tumor as the source of laminin and type IV collagen. Previous investigators have demonstrated that antigens expressed on type IV collagen and laminin isolated from the EHS tumor matrix cross-react with normal human and murine basement membranes (4, 15, 22, 23). C57BL/6 mice were used in the present studies for both passage of the EHS tumor line and as hosts for laminin sensitization.

The basis for cell-mediated immunity

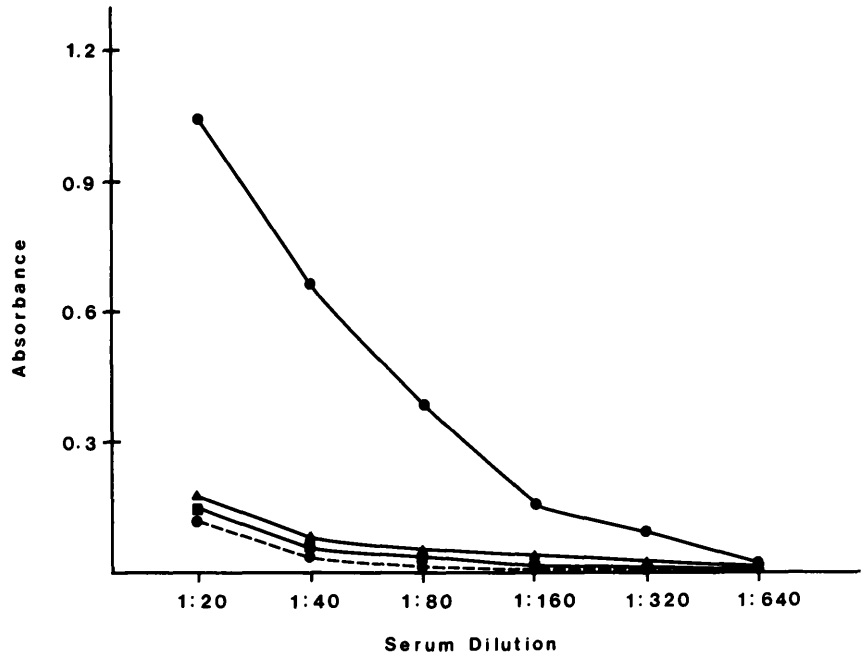

FIG. 3. Analyses of immune and normal mouse sera on laminin-coated wells using the ELISA. Serial dilutions of mouse anti-laminin sera (●), mouse anti-type I collagen sera (▲), mouse anti-type IV collagen sera (■), and normal mouse sera (– – –) were assayed on microtiter wells coated with 1.25 μg laminin. The results are expressed as the mean absorbance value at 414 nm.

(CMI) to connective tissue components was established earlier by Adelmann and coworkers (24, 25) and by Senyk and Michaeli (26). These investigators demonstrated that type I collagen could serve as an antigen for the induction of a delayed-type hypersensitivity (DTH) response; however, they reported conflicting results regarding the ability of an animal to mount an immune response to homologous collagens. Senyk and Michaeli (26) demonstrated DTH skin reactions in guinea pigs to homologous type I collagen. However, Adelmann *et al.* (24, 25) were unable to demonstrate CMI in guinea pigs to homologous type I collagen employing similar techniques. These observations may be attributable to the strain differences of experimental animals.

Our data demonstrate the ability of homologous laminin to induce cell-mediated and humoral immunity in a murine system. Mice receiving a single sc injection of 5 μg laminin in FCA developed significant footpad swelling 24 hr after challenge with laminin. This DTH response reached a maximal level on Day 7 postsensitization. The response was specific for laminin and was not observed after challenge with type I collagen, type IV collagen, or fibronectin. Histological examination of the footpad lesion revealed an infiltration of mononuclear cells, indicative of a classical DTH response. Laminin-sensitized spleen cells adoptively transferred DTH responsiveness to normal syngeneic mice and depletion of T cells by prior treatment of the immune spleen cells with anti-Thy 1.2 serum plus C eliminated the ability to transfer this response. Thus, mice sensitized to laminin mount an antigen-specific DTH response to this basement membrane antigen.

Although antibody was not detected in mice displaying DTH to laminin after a single injection, higher concentrations of the sensitizing antigen and use of protocols employing repeated immunization elicited significant antibody titers to laminin. No cross-reactivity was exhibited by antisera

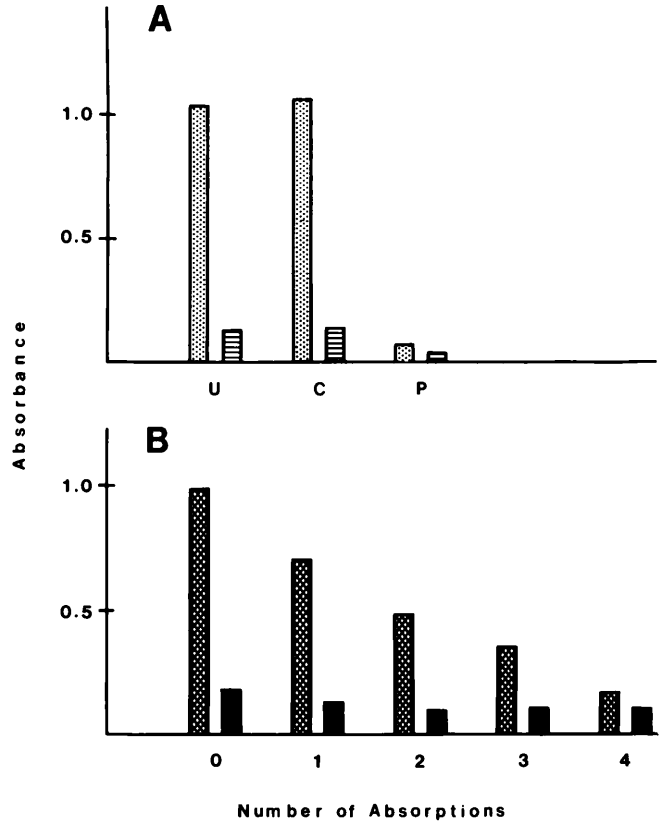

FIG. 4. **(A)** Reactivity of normal (▤) and anti-laminin (▦) mouse sera on microtiter wells coated with 1.25 μg (U) untreated laminin, (C) collagenase-treated laminin, and (P) Pronase-treated laminin preparations. **(B)** Repeated absorptions of anti-laminin (▩) and normal (■) mouse sera on laminin-coated wells. All sera were assayed at a 1:20 dilution on wells coated with 1.25 μg laminin. The results are expressed as indicated in Fig. 3.

to types I or IV collagen assayed with laminin, nor did laminin immune sera react with other connective tissue components. Collagenase treatment of laminin prior to analysis in the ELISA showed no alteration in reactivity of the sera with laminin.

Although the potential of basement membrane to act as an autoantigen has been shown, the precise antigens involved are in most instances unknown. Cell-mediated and/or humoral immunity to basement membranes could be directed at either collagenous or noncollagenous components, or both. Data from our laboratory support the hypothesis that basement membrane damage could be initiated or perpetuated by immunity to basement membrane collagen (13, 14). Significant titers of anti-type IV collagen antibodies were detected in the sera of some patients with scleroderma. Antibody levels correlated inversely with pulmonary diffusion capacity, a reliable early indicator of pulmonary interstitial disease. These anti-collagen autoantibodies were primarily of the IgM class, an immunoglobulin that is a potent activator of the complement cascade (13). Gay *et al.* (27) have reported autoantibodies to a collagenous component of basement membrane (C chain) in the sera of patients with epidermolysis bullosa simplex, a severe blistering skin disease. We have previously reported the induction of cell-mediated and humoral immunity to isologous type IV collagen in a

murine model (11, 13). Foidart *et al.* (28) demonstrated anti-laminin and anti-type IV procollagen antibodies in the sera of patients with Goodpasture's syndrome. Szarfman *et al.* (29) have reported that sera from humans with Chagas' disease and Rhesus monkeys infected with *Trypanosoma cruzi* contain antibodies which react with laminin. Furthermore, injection of affinity-purified sheep anti-laminin IgG into rabbits results in antibody binding to the glomerular basement membrane, with subsequent alterations in glomerular structure and proteinuria (30). These studies indicate that immunity to basement membrane components may be involved in disease processes.

Changes in vascular basement membranes occur during the progression of diseases such as scleroderma, atherosclerosis, and diabetes mellitus. Alterations in basement membrane structure and/or assembly may reflect endothelial damage, which may result in exposure of the basement membrane and induction of immunity to previously sequestered basement membrane components. Our observations and those of Foidart *et al.* (28) and Szarfman *et al.* (29) suggest that laminin, the major noncollagenous glycoprotein of basement membranes, is a strong immunogen, capable of inducing cell-mediated and humoral immunity. Studies are currently underway to investigate the possible immunopathological sequelae which may occur following the induction of immunity to homologous laminin in mice. The nature of the body components which elicit an immune response (autoimmunity) in connective tissue diseases is the subject of continuing investigation. The development of an animal model based on laminin immunity may lead to a better understanding of the inflammatory responses which occur in human and experimental animal diseases involving basement membranes.

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