Autoimmune Vasculitis Resulting From In Vitro *Immunization of Lymphocytes to Smooth Muscle*

MICHAEL N. HART, MD, SANDRA K. TASSELL, MS, KATHRYN L. SADEWASSER, BS, ROBERT L. SCHELPER, MD, PhD, and STEVEN A. MOORE, MD, PhD

From the Department of Pathology, Division of Neuropathology, University of Iowa College of Medicine, Iowa City, Iowa

Lymphocytes sensitized *in vitro* to syngenic microvascular smooth muscle and transferred to syngeneic recipients produced *in vivo* microvessel vasculitis characterized by mononuclear cells which adhered to endothelium, infiltrated the vessel wall, and formed a perivascular cuff. A granulomatous type of vascular inflammation was seen in 20% of the affected recipients in which the vessel smooth muscle appeared to be preferentially attacked. These lesions bear a striking resemblance to certain human vasculitides, and the model provides an important means of studying vasculitis as well as general cellular autoimmune disease mechanisms. (Am J Pathol 1985, 119:448–455)

HUMAN VASCULITIS is a fairly common disease entity. It is often an autoimmune disorder which can selectively involve blood vessels or can occur in association with other diseases, notably the "collagen vascular" diseases such as lupus erythematosus, rheumatoid arthritis, and dermatomyositis. Immune complex deposition has been observed in a few types of vasculitis and is probably the cause of those vasculitides in which it is seen.[1] Direct, lymphocyte-mediated vasculitis has also been hypothesized, but not proven. Its existence is suggested by lymphocytic infiltrates of vessels in many vasculitides, particularly those vasculitides associated with the collagen vascular diseases.[2]

The development of a model of cellular autoimmune vasculitis would not only show that a lymphocyte-mediated pathogenesis of vasculitis is possible in humans but would also allow studies of the mechanisms involved in general and specific (vasculitis) cellular autoimmune diseases. Although cellular autoimmunity is a much discussed entity, there are only a few promising models for its study, few of which utilize an *in vitro* immunization technique.[3] We now report a new model for autoimmune vasculitis in which lymphocytes are cultured *in vitro* with syngeneic microvascular smooth muscle (SM) and then injected into syngeneic recipients, with resultant inflammatory lesions of microvessels.

We previously reported a similar model in which lymphocytes were co-cultured with allogeneic endothelium and injected into mice syngeneic to the lymphocytes.[4] The current model differs from the previous model in three ways: first, the co-culture substrate cells are SM; second, SM is syngeneic both to the co-cultured lymphocytes and to the recipient mice, which makes the model completely autoimmune in nature; and third, a granulomatous-type vasculitis that was never seen in the endothelial model develops in 20% of the recipient mice.

Material and Methods

Animals

All animals used were female BALB/c mice between 2 and 4 months of age obtained from Simonson Laboratories (Gilroy, Calif). The mice are regularly checked for histocompatibility by H-2 typing and by skin grafting. They do not develop spontaneous vasculitis. All injections were intravenous.

Supported by Veterans Administration Research Funds, NIH Grant HL 14230 (Arteriosclerosis Specialized Center of Research sponsored by The National Heart, Lung and Blood Institute), and NIH Grant HL 31944.

Accepted for publication January 28, 1985.

Address reprint requests to Michael Noel Hart, MD, University of Iowa College of Medicine, Department of Pathology, 105 ML, Iowa City, Iowa 52242.

Smooth Muscle Isolates

Lines of BALB/c microvascular SM were developed in our laboratory by straining an emulsion of mouse brain through a 153-μ mesh nylon filter which traps small, muscular blood vessels. The microvessels are plated onto plastic dishes in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, New York) with 20% fetal calf serum (FCS) (KC Biological Co., Lenexa, Kan) and treated for 2 minutes with 1 mg/ml of collagenase (Worthington Biochemical, Freehold, NJ). Cells migrate from these vessels and proliferate sufficiently to be passaged in 2–3 weeks. Analysis of the α-actin content of the cells and electron-microscopic studies have shown that they are virtually all vascular SM. Complete details of the technique are given in a separate publication.[5] The monolayers used in these experiments were all within 10 passages.

Co-cultures

Splenic lymphocytes from BALB/c mice were harvested under sterile conditions and separated from other splenic cellular elements by a standard Ficoll technique.[6] Co-cultures of BALB/c splenic lymphocytes with low-passage BALB/c SM were carried out in RPMI 1640 medium (GIBCO) with 20% FCS (KC Biological) 5×10^{-5} M 2-mercaptoethanol, 0.2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in T-75 flasks (Corning Glass Works, Corning, NY) at an average ratio of 8:1 (lymphocytes/SM). The medium was changed every 2–3 days. After 7 days of co-culture the lymphocytes were separated from the SM by gentle agitation with 0.02% EDTA in phosphate-buffered saline. Prior to intravenous injection into recipients, the percentage of activated lymphocytes was determined by counting the number of large lymphocytes resembling blasts on a hematoxylin and eosin (H&E)-stained smear and dividing by the total number of lymphocytes counted. Lymphocytes cultured in RPMI 1640 medium in the absence of SM became nonviable within 48 hours, as determined by 1% Evans blue dye exclusion tests.

Tissue Studies

The recipients underwent autopsy at intervals of from 6 to 14 days with two or three sections from each animal taken routinely of brain, lungs, liver, kidneys, skeletal muscle, thymus, spleen, and gastrointestinal tract; there was only occasional sampling of heart, pancreas, great vessels, uterus, bladder, and lymph nodes, because these latter organs never showed lesions. Most recipients underwent autopsy at 6–8 days, and approximately 10 were autopsied at 14 days. Previous experience had shown that lesions do not develop earlier than 2–3 days and are maximal at about 1 week.[4] The number of and severity of lesions were scored 1–4+, with 1+ indicating at least 2 vessels involved per animal, 2+ indicating 3–5 vessels, 3+ indicating 6–15 vessels, and 4+ indicating more than 15 vessels involved. Involvement of a vessel was defined as either transmural involvement by lymphocytes or definite evidence of mural destruction. Perivascular cuffing was not counted.

Experiments With Cyclophosphamide

Cyclophosphamide (Cy) (Meade Johnson & Co., Evansville, Ind) was injected into series of donor and recipient mice in order to enhance the development of the lesions. In one series, the donors of the splenic lymphocytes were given 25 mg/kg of Cy 2–3 days before donation to effect suppression.[7,8] After co-culture, these lymphocytes were injected into 21 recipients. In another series of 41 recipient mice, both donor and recipients received Cy (25 mg/kg to donors; 200 mg/kg to the recipients). Cy was given to the recipients 7 days prior to injection. An additional 10–12 mice in this series died prematurely from the Cy treatment and were not included in the study.

Controls

These consisted of 1) injection of normal lymphocytes into normal (51) and Cy-treated (33) recipients, 2) lymphocytes activated by co-culture with syngeneic skeletal muscle and injected into normal (28) and Cy-treated (52) recipients, and 3) splenic lymphocytes activated nonspecifically by incubation for 72 hours with 8.0 μg/ml of phytohemagglutinin (Miles Laboratories, Elkhart, Ind) (8 recipients) or for 72 hours with 2 μg/ml of concanavalin A (Sigma Chemical Co., St. Louis, Mo) (8 recipients). In addition, 8 mice were given 200 mg/kg of Cy and underwent autopsy. Six mice were each given approximately 1.2×10^6 SM cells and underwent autopsy. All of these control animals were autopsied at 7 days after lymphocyte injection.

We injected 3-day co-culture medium without cells into 10 mice (0.2 ml each) to see whether soluble factors could elicit vasculitis. An additional 5 mice were given 0.2 ml of fresh medium alone as a further control. These groups of animals were autopsied at 5 days after injection.

Other Experiments

Preliminary experiments performed in our laboratory have addressed some of the mechanisms involved

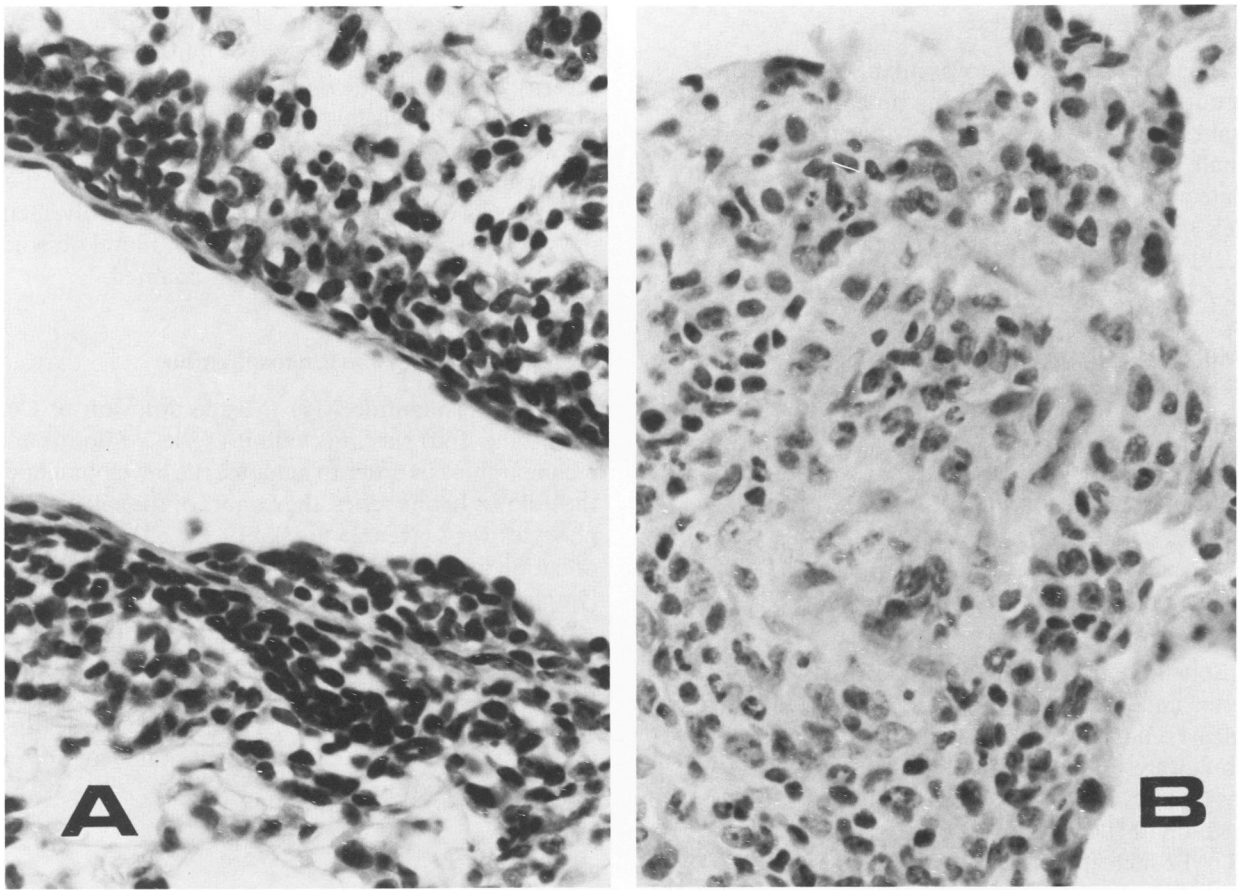

Figure 1A—A thin-walled microvessel in the lung showing lymphocytes cuffing the vessel, adhering to the endothelium, and infiltrating through the wall. This type of lesion is the predominant one seen in this model. (H&E, × 100) **B**—A pulmonary arteriole displaying a granulomatous-like vasculitis with infiltration of lymphocytes, macrophges, and occasional eosinophils and vessel-wall destruction. This type of vasculitis is present in 20% of the affected recipients. (H&E, × 100)

in the pathogenesis of this autoimmune vasculitis. In one experiment, we irradiated lymphocytes (2000 rads) after co-culture but before injection into 6 mice to see whether nonproliferating cells could cause the lesions.

Phenotyping was performed on a few lesions for the Ly-1 and Ly-2,3 surface antigens with 10 μ frozen sections fixed for 2 minutes in cold 100% ethanol on glass slides. Commercial monoclonal rat anti-Ly antibodies (Becton-Dickinson, Mountain View, Calif) were used in an indirect fluorescent (FCA) labeling system. The primary antibody was diluted 1:50 and secondary, fluorescein-conjugated goat anti-rat IgG was diluted 1:100. The lesions were also screened for the presence of immunoglobulin with fluorescein-conjugated goat anti-mouse IgG, IgA, and IgM (N. L. Cappel Laboratories, Malvern, Pa) at a 1:50 dilution on 10-μ ethanol-fixed frozen sections.

In all test and control co-cultures we used 20% FCS in the RPMI 1640 medium. In order to address the possibility that the lymphocytes in our co-culture system were recognizing FCS complexed with SM membrane antigens as foreign, we co-cultured lymphocytes with SM in the absence of FCS but in the presence of 0.5% BALB/c serum.

In an experiment designed to localize the lymphocytes after injection into the recipients, 6×10^6 co-cultured, sensitized lymphocytes were labeled with a 60-minute pulse of 100 μCi of ^{51}Cr (New England Nuclear, Boston, Mass) in a 1-ml cell suspension, followed by one wash in Hanks' balanced salt solution (HBSS), resuspension in HBSS, and injection of 6.6×10^5 cells into each of 9 recipients. The recipients underwent autopsy at 2 and 24 hours, 10-μ sections were coated with a 1:1 dilution of emulsion (Kodak Nuclear Track Emulsion Type NTB, Eastman Kodak, Rochester, NY) in distilled water, exposed at 4 C for 2 weeks, and developed in Dektol (Eastman Kodak) for 2 minutes at 15 C.

Results

Tissue Studies

In 29% of the initial test animals vasculitis developed, characterized by adherence of mononuclear cells onto the endothelium, with invasion of the media and ad-

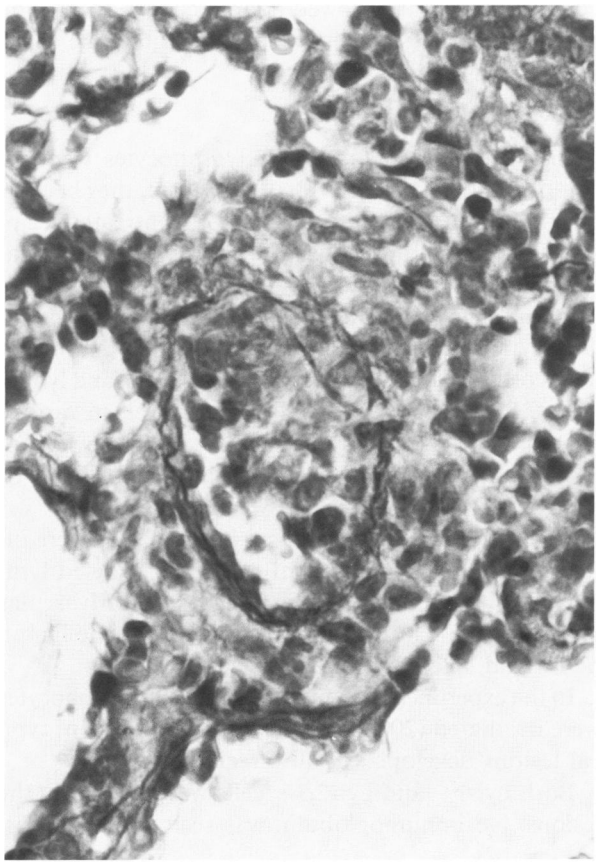

Figure 2—Granulomatous inflammation of a small vessel showing fragmentation of elastica (*dark lines*) and medial destruction. (Elastic–Van Gieson, × 160)

ventitia of arterioles and venules (Figure 1A). Plasma cells, eosinophils, and/or polymorphonuclear cells were found in about 10% of the lesions. Lesions were most commonly seen in the lungs and liver, but they were found to varying degree in every organ sampled except brain parenchyma, gastrointestinal tract, and great vessels. Most of the animals were scored as 1+ or 2+. There was no apparent relationship between the score and the pattern of involvement, the use or non-use of Cy, irradiation of lymphocytes, or the severity of the lesions. The lesions developed 2–3 days after cell transfer, were maximal at 7–10 days, and abated by 14 days. Twenty percent of the affected test animals showed, in addition to the above described type of vasculitis, a granulomatous vasculitis of pulmonary arterioles characterized by obliteration of the lumen and involvement of the media by lymphocytes, macrophagelike cells, and eosinophils (Figures 1B and 2). A few vessels in random animals showed what appeared to be selective inflammation and destruction of the media which was not granulomatous (Figure 3). With the exception of the granulomatous lesions and the medial inflammation, the vasculitis seen in this model is morphologically identical to that seen in our earlier model, in which lymphocytes were co-cultured with endothelium. Neither granulomatous lesions nor selective medial inflammation were ever seen in that model.[4]

Cy Experiments

Injection of Cy into donor mice to kill suppressor cells and injection of Cy into both donors and recipients

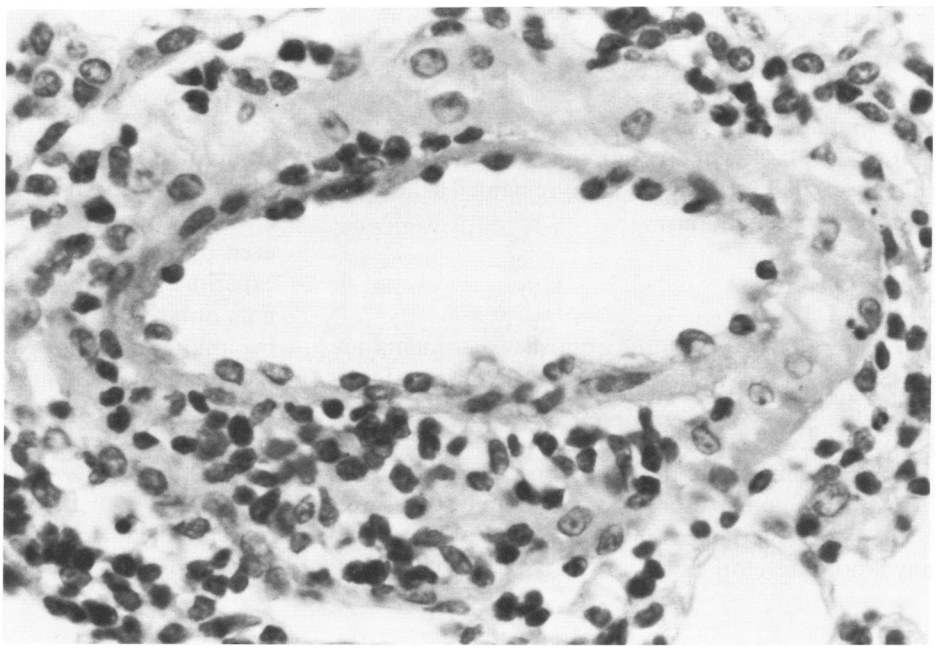

Figure 3—Selective medial (*smooth muscle*) inflammation in a pulmonary arteriole. (H&E, × 160)

Table 1

Type of lymphocyte injected	% and number of recipients with lesions
Test*	
1. Lymphocytes co-cultured with SM	29% (15/51)
2. Lymphocytes from donor mice that were given low dose (25 mg/kg) Cy before co-culture	52% (11/21)
3. Lymphocytes from Cy-treated donors (25 mg/kg) before co-culture; recipients treated with Cy (200 mg/kg) before injection	71% (29/41)
Control†	
1. Fresh unactivated lymphocytes injected into normal mice	0% (0/51)
2. Fresh unactivated lymphocytes injected into Cy-treated (200 mg/kg) recipients	0% (0/33)
3. Lymphocytes co-cultured with syngeneic skeletal muscle	0% (0/28)
4. Lymphocytes co-cultured with syngeneic skeletal muscle and recipients treated with 200 mg/kg Cy	11% (6/52)
5. Lymphocytes activated by	
a. Concanavalin A	0% (0/8)
b. Phytohemagglutinin	0% (0/8)
Other controls	Results
6. 8 mice given 20 mg Cy	Questionable abnormalities of endothelium
7. 10 mice each given 0.2 ml supernatant from co-culture and 5 mice each given 0.2 ml RPMI 1640 medium alone	One lesion in one mouse
8. 6 mice each given 1.2 × 10^6 SM cells intravenously	No lesions

* The use of Cy in the donor mice, followed by its use in both donor and recipient, progressively increased the number of recipients with lesions. Of the 55 test mice with lesions, 11 (20%) showed granulomatous vasculities.

† Results of the control experiments. In the 6 mice in which lesions developed in Control 4, there was no detectable differnce between these lesions and those of the test mice in terms of distribution, cellular makeup, or severity.

correspondingly increased the percentage of mice with lesions (see Table 1), although the percentage of mice showing granulomatous vasculitis remained at 20% after these manipulations.

Controls

The results of all of the control experiments are shown in Table 1. Six of 52 Cy-treated recipients of control lymphocytes activated to skeletal muscle developed lesions that were no different in character, severity, location or score distribution from those of the test mice. Two of these control mice had granulomatous lesions.

In none of the 5 mice that received fresh medium did any lesions develop, but 1 of the 10 mice that received co-culture medium showed a large collection of lymphocytes in a lung with mural involvement of one vessel.

Other Experiments

In the experiment in which lymphocytes were co-cultured with SM in 0.5% BALB/c serum, they became blastic, and vasculitis resulted in 2 of 5 untreated recipient mice, indicating that the lymphocytes were probably not being immunized (sensitized) to FCS.

Animals given 200 mg/kg of Cy either alone or with fresh lymphocyte transfer showed marked, but not total, depletion of lymphocytes in their spleens and lymph nodes. Endothelium of small blood vessels in various sporadic locations of many of these mice showed apparent abnormalities of endothelium consisting of edema and/or hypertrophy by light microscopy.

When ^{51}Cr-labeled, activated lymphocytes were injected into untreated normal recipients, labeled lymphocytes were found at 2 and 24 hours in perivascular locations in the lung and liver as well as in the lymph nodes and spleens of the recipients.

In the experiment in which the activated lymphocytes were irradiated (2000 rads) just before injection, typical lesions developed in all 6 recipient mice.

Both Lyt-1+ and Lyt-2,3+ cells were present in the lesions. No immunoglobulin was seen in the lesions.

Discussion

The results of this study show that co-culture of mouse lymphocytes with microvascular smooth muscle in a totally syngeneic system results in vasculitis. The vasculitis is predominantly mononuclear and in most recipients does not differ from that reported previously in a lymphocyte–endothelium co-culture model.[4] However, 20% of the recipients in the present experimental model developed a granulomatous-appearing vasculitis which was never seen in the endothelial model. The granulomatous-appearing vasculitis in this model is similar in appearance to that reported by Johnson et al, after injection of glucan particles into rats.[9] However, particulate matter was never seen in the present experimental lesion after examination by either ordinary or birefringent light. The granulomatous-appearing lesions also have a morphologic appearance which is very similar to that seen in the allergic angiitis and granulomatous types of vasculitis in humans.[10] There is no apparent relationship between the number and the site or intensity of lesions in any given animal with the use of Cy or irradiation of donor cells. This may reflect individual idiosyncrasies of the animal's immune system, or it may reflect inadequacies in our sampling

system. The end point of our experiments is a biologic assay (vasculitis), and it is logistically (and financially) impossible to exhaustively section each organ of each animal. The ability to do so would not result in a decrease in the number of affected animals.

Irradiation of sensitized lymphocytes prior to injection does not hinder the development of lesions. This observation, in accompaniment with the observation that labeled lymphocytes initially lodge in areas where vasculitis is subsequently observed, suggests that injected, sensitized lymphocytes locally recruit host lymphocytes to mediate the lesions. On the other hand, experiments in which the recipient's immune system was severely compromised by Cy resulted in large numbers of recipients with lesions. This indicates that the injected cells may also proliferate *in situ*. It may also indicate that 200 mg/kg Cy in these animals was not a sufficient dose to functionally ablate their immune system. This possibility is supported by our observation of subtotal lymphocyte depletion in animals given only 200 mg/kg of Cy. The injection of lymphocytes into Cy-treated recipients may also help to reconstitute the system. In addition, high doses of Cy may injure the vascular wall in such a way as to enhance the deposition of lymphocytes. Giving low doses of Cy to the donors probably increased the percentage of animals with lesions by selectively killing suppressor cells, since this result of Cy treatment has been demonstrated in other systems.[7,8]

The phenotype experiments are preliminary, but they do indicate that both helper/inducer (Lyt-1+) and cytotoxic/suppressor (Lyt-2,3+) lymphocytes are present in the lesions.[11] Further experiments must elucidate which of these phenotypes arrives first, whether the Ly+2+3+ lymphocytes are cytotoxic or suppressor cells, and what the respective roles of eosinophils, plasma, cells and macrophagelike cells are in the lesions. Further search for immunoglobulin in the lesions must be undertaken also. It may have an evanescent role in the development of the lesion.

Six (11%) of the control animals given injections of skeletal muscle co-cultured lymphocytes developed typical lesions. This may indicate some sharing of surface antigens between SM and skeletal muscle. It may also indicate that one of the effects of Cy is to injure the vessel wall in such a way that it is made more permeable to activated lymphocytes. Again, noteworthy in this regard was our finding of endothelial changes in control mice given 200 mg/kg of Cy alone. One control animal out of 10 that received co-culture supernatant developed a lesion in one vessel. Whether this was a spurious finding due to some other cause or a result of the supernatant is not known. We plan to address this question by injecting more mice with concentrated supernatant. The other control experiments indicate that injected SM or nonspecifically activated lymphocytes do not elicit the lesions.

There is a precedent for the activation (immunization) of lymphocytes to syngeneic tissue *in vitro*. Cohen and Wekerle[12] have shown that rat lymphocytes activated by co-culture with syngeneic reticulum or fibroblasts will, in turn, lyse syngeneic reticulum or fibroblast targets *in vitro*. More recently, Charreire and Michel-Bechet[13] successfully developed autoimmune thyroiditis by co-culturing mouse lymphocytes with syngeneic thyroid epithelial cells and then either directly injecting the sensitized lymphocytes into the thyroid or injecting them intravenously. They also demonstrated anti-thyroid immunoglobulins in their model, indicating that their sensitized lymphocytes activate B cells in addition to presumably attacking the thyroid directly. These studies raise questions regarding the nature of the surface antigens on the stimulator cells that are recognized by the lymphocyte. It is known that human or animal lymphocytes will proliferate when mixed with syngeneic lymphocytes (autologous mixed lymphocyte reaction, AMLR) and that the stimulus to proliferation is the presence of Class II major histocompatibility (Ia) antigens on the surface of some of the cells in the mixture.[14] It is currently believed that T-lymphocytes can recognize either Ia antigens alone or foreign antigens in association with Ia determinants. On the one hand, evidence derived via the AMLR suggests that lymphocytes proliferate in response to Ia determinants alone.[15] On the other hand, under most human disease and experimental conditions it is most plausible that T-lymphocytes recognize and respond to foreign antigens in conjunction with self-Ia. This has been shown to be true in *in vitro* systems[16] as well as in organ transplantation models where host T-lymphocytes may react to specific antigenic determinants shared by endothelial cells and macrophages.[17,18] Autoimmunity, however, is extremely complex, and several pathogenic mechanisms may be operative. These include aberrancies of antigen presentation to T-lymphocytes, defects in suppressor mechanisms, responsiveness to foreign antigens with cross-reactivity to self-antigens, and failure to develop tolerance in neonatal life.[19,20] Most autoimmune diseases are antibody-mediated, although abnormalities of regulatory T-lymphocytes may play the most significant roles in many of these diseases.[19]

In the currently reported model, it can be hypothesized that lymphocytes recognize endogenous SM antigens in context with Ia. The endogenous SM antigen would be recognized as foreign by the lymphocytes because they do not normally "see" vascular SM. Although nothing is known regarding the antigen-

presenting properties of SM, it is well known that endothelium expresses antigens more or less unique to endothelium[21,22] and variably expresses Ia determinants.[23,24] Endothelium also appears to be able to substitute for macrophages in the presentation of antigen.[25] Perhaps SM also possess similar properties. The finding of SM antibodies in humans under various circumstances suggests that some types of SM may have unique surface antigens.[26] Further support for this hypothesis comes from the observation in our model in which vascular SM appears to be preferentially attacked. Preliminary work in our laboratory suggests that our SM cells display Class II antigens (IA and IE). Future experiments will further address this observation.

In addition to the significance of Ia determinants in activating immunocompetent cells, there may be a very important role for these determinants at the effector level. This is underscored by the observation of attenuation of experimental autoimmune diseases after injection of anti-Ia,[27] although it is not clear whether the action of these antibodies is at the target level or whether they interfere with immune regulation.[27,28]

Why normal controls against autoimmunity are apparently lost when lymphocytes have been co-cultured with syngeneic tissues *in vitro* is not known. Further investigation into this question might provide valuable clues to the pathogenesis of autoimmune diseases.

It is also not known why the brain is not involved in this model, in spite of the fact that our smooth muscle is brain-derived. Possibly the cerebral endothelium excludes the activated lymphocytes as one of the facets of central nervous system "immune privilege."[28]

This model of autoimmune vasculitis offers promise for several reasons: 1) The lesions seen in the model strongly resemble those of certain human vasculitides, particularly those associated with the collagen vascular diseases and the granulomatous vasculitides. 2) The model also mimics human autoimmune disease inasmuch as some individuals get the disease and some do not—depending upon poorly understood immune regulatory mechanisms. 3) The *in vitro* methodology of the model offers a system in which immunologic regulatory mechanisms can be easily manipulated. 4) Antigen-presenting properties of vascular SM may play a role in the pathogenesis of arteriosclerotic lesions.

References

1. Tappeiner G, Jordan RE, Wolff K: Circulating immune complexes in necrotizing vasculitis, Vasculitis. Edited by K Wolff, RK Winkelman. London, Lloyd-Luke, 1980, pp 68–75
2. Cupps TR, Fauci AS: Pathophysiology of vasculitis, Major Problems in Internal Medicine, Vol 21, The Vasculitides. Edited by LH Smith. Philadelphia, W. B. Saunders, 1981, p 17
3. Cohen IR, Ben-Nun A, Holoshitz J, Maron R, Zerubavel R: Vaccination against autoimmune disease with lines of autoimmune T lymphocytes. Immunol Today 1983, 4: 227–230
4. Hart MN, Sadewasser KL, Cancilla PA, DeBault LE: Experimental autoimmune type of vasculitis resulting from activation of mouse lymphocytes to cultured endothelium. Lab Invest 1983, 48:419–427
5. Moore SA, Strauch AR, Yoder EJ, Rubenstein PA, Hart MN: Cerebral microvascular smooth muscle in tissue culture. In Vitro (In press)
6. Boyum A: Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 29 (Suppl 97):9
7. Rollinghoff M, Starzinski-Powitz A, Pfizenmaier K, Wagner H: Cyclophosphamide-sensitive T lymphocytes suppress the in vivo generation of antigen-specific cytotoxic T lymphocytes. J Exp Med 1977, 145:455–459
8. Askenase PW, Hayden BJ, Gershon RK: Augmentation of delayed-type hypersensitivity by doses of cyclophosphamide which do not affect antibody responses. J Exp Med 1975, 141:697–702
9. Johnson KJ, Glovsky M, Schrier D: Animal model of human disease: Pulmonary granulomatous vasculitis induced in rats by treatment with glucan. Am J Pathol 1984, 114:515–516
10. Cupps TR, Fauci AS: Systemic necrotizing vasculitis of the polyarteritis nodosa group,[2] pp 44–49
11. Swain SL, Dutton RW: Mouse T-lymphocyte subpopulations; relationships between function and Lyt antigen phenotype. Immunol Today 1980, 1:61–65
12. Cohen IR, Wekerle H: Autosensitization of lymphocytes against thymus reticulum cells. Science 1972, 17:1324–1325
13. Charreire J, Michel-Bechet M: Syngeneic sensitization of mouse lymphocytes on thyroid epithelial cells: III. Induction of thyroiditis by thyroid-sensitized T lymphocytes. Eur J Immunol 1982, 12:421–425
14. Glimcher LH, Longo DL, Green I, Schwartz RH: Murine syngeneic mixed lymphocyte response: I. Target antigens are self Ia molecules. J Exp Med 1981, 154: 1652–1670
15. Hausman PB, Stites DP, Stobo JD: Antigen-reactive T cells can be activated by autologous macrophages in the absence of added antigen. J Exp Med 1981, 153:476–481
16. DeFreitas EC, Chestnut RW, Grey HM, Chiller JM: Macrophage-dependent activation of antigen-specific T cells requires antigen and a soluble monokine. J Immunol 1983, 131:23–29
17. Hirschberg H, Braathen LR, Thorsby E: Antigen presentation by vascular endothelial cells and epidermal Langerhans cells: The role of HLA-DR. Immunol Rev 1982, 66:57–77
18. Sprent J, Korngold R: H2 restriction of T cells mediating lethal graft-versus-host disease to minor histocompatibility determinants. Adv Exp Med Biol 1981, 49:531–536
19. Theofilopoulis AN, Dixon FJ: Autoimmune diseases: Immunopathology and etiopathogenesis. Am J Pathol 1982, 108:321–365
20. Fischbach M: Defective T cell response to presented antigen in autoimmune mice. J Immunol 1984, 133:2365–2368
21. Darnule AT, Stotzky G, Darnule TV, Turino GM, Mandle I: Identification of surface antigens on endothelial cells. Immunol Commun 1983, 12:351–362
22. Cui YC, Tai PC, Gatter KC, Mason DY, Spry CJF: A vascular endothelial cell antigen with restricted distribution in human foetal, adult and malignant tissues. Immunol 1983, 49:183–189

23. Hinglais N, Kazatchkine S, Charron D, Appay MD, Mandet C, Paing M, Bariety J: Immunohistochemical study of Ia antigen in the normal and diseased human kidney. Kidney Int 1984, 25:544–550
24. Rosa F, Fellows M: The effect of gamma-interferon on MHC antigens. Immunol Today 1984, 5:261–262
25. Hirschberg H, Bergh OJ, Thorsby E: Antigen-presenting properties of human vascular endothelial cells. J Exp Med 1980, 152:249–255
26. Toh BH: Smooth muscle autoantibodies and autoantigens. Clin Exp Immunol 1979, 38:621–628
27. Sriram S, Steinman L: Anti Ia antibody suppresses active encephalomyelitis: Treatment model for diseases linked to IR genes. J Exp Med 1983, 158:1362–1367
28. Steinberg AD, Santoro TJ: Anti-Ia antibodies: A revolutionary therapy for immune-mediated diseases? Immunol Today 1984, 5:13–14
29. Darling JL, Hoyle NR, Thomas DGT: Self and non-self in the brain. Immunol Today 1981, 2:176–181

Acknowledgments

We thank E. Yoder, C. TenEyck, E. Witters, K. Hansen, and L. Van Dyk for technical help and H. Urnovitz, R. Lynch, G. Milburn, J. Kemp, and S. Binion for advice.