

MECHANISMS AND SUPPRESSION OF INFLAMMATORY DEMYELINATION

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Abstract. Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system commonly used as a model for multiple sclerosis. In both of these diseases demyelination occurs in association with perivascular infiltrates of T-cells and macrophages. The similarities in immunopathology suggest that these two diseases share common mechanisms of tissue destruction. We have proposed a general mechanism to explain the clinical and histopathological features of EAE. T-cells sensitized to the inducing antigen, myelin basic protein (MBP), react with antigen-presenting cells (possibly endothelial cells, microglia or astrocytes) in the central nervous system. As a consequence of this reaction, T-cells release lymphokines which activate macrophages, stimulate an augmenting inflammatory response, and, through the action of vasoactive amines, induce vasospasm and breakdown of the blood-brain barrier. The activated macrophages secrete inflammatory mediators, including plasminogen activator and other proteinases, which, in concert with serum plasminogen and complement, initiate myelin destruction. The macrophage products also serve to enhance the inflammatory response and vascular permeability. In support of this hypothesis we find that: (1) macrophage-secreted proteinases can degrade

MBP in lyophilized myelin and that proteolysis is amplified in the presence of plasminogen; (2) proteolysis of proteins in fresh myelin by macrophage proteinases and plasminogen or by plasmin is potentiated by complement; (3) removal of macrophages from the circulation suppresses EAE; (4) proteinase inhibitors suppress EAE; and (5) prazosin, an α_1 -adrenergic receptor antagonist, suppresses the clinical signs of EAE and the increased vascular permeability but only delays the inflammatory response. We believe that prazosin acts on the vascular α_1 -adrenergic receptor to inhibit vasospasm and prevent opening of the blood-brain barrier. Thus it is possible to suppress both clinical signs and pathology by interceding at several steps of the cell-mediated immune reaction.

INTRODUCTION

Multiple sclerosis (MS) and the animal model disease, experimental autoimmune encephalomyelitis (EAE), are both characterized by perivascular inflammation, edema, breakdown of the blood-brain barrier, demyelination and reactive gliosis. It is known that EAE involves cell-mediated immune (CMI) mechanisms requiring T-cell sensitization to myelin basic protein, a central nervous system (CNS) antigen. The formation of the MS lesion, which contains T-lymphocytes and macrophages, is also generally acknowledged to be related to a CMI response, although the antigen(s) responsible have not been identified. For the past dozen years we have used EAE to explore mechanisms by which such CMI reactions might initiate inflammation, edema and demyelination, and how such reactions might be suppressed.

POSSIBLE ROLE OF ACTIVATED MACROPHAGES

The generally accepted sequence of cellular reactions in a CMI response is that sensitized lymphocytes invade the tissue and react with the antigen on an antigen-presenting cell; this reaction stimulates T-cells to release factors known as lymphokines; these factors "call in" monocytes from the circulation and stimulate them, as well as resident macrophages, to undergo a marked metabolic change known as activation, and thus to become fully differentiated macrophages.

Macrophages are a major component of the perivascular cuffs in both MS and EAE (1, 2, 24). In EAE cellular infiltration precedes demyelination, which is observed only in areas where macrophages are present. These cells can be seen penetrating between myelin lamellae (17, 25), but other observations suggest that macrophages can also damage nearby myelin sheaths without actually contacting them. For example in EAE extensive vesicular disruptions of myelin are seen in proximity to ma-

crophages (12, 25). Similar observations in MS are the net-like disruptions of myelin, the apparent melting away of myelin in contact with macrophages, and the presence of myelin debris in the extracellular space as well as within macrophages (23, 24).

These morphological observations took on more significance with the discovery that activated macrophages secrete a number of products that mediate inflammation and are capable of tissue destruction. Among these are several neutral proteinases, including plasminogen activator (31). It was also known that both acid and neutral proteinases are elevated in MS plaques and EAE lesions (see 22 and 29 for reviews).

The data summarized above led to our hypothesis that inflammatory demyelination could be initiated by secretion products of activated macrophages (10). We proposed that infiltrating sensitized T-cells "called in" and activated macrophages, which would secrete plasminogen activator. Since the blood-brain barrier is destroyed at the site of the lesion, serum proteins, including plasminogen, would be present in the CNS parenchyma. The plasminogen activator could catalyze the formation from plasminogen of plasmin, a trypsin-like neutral proteinase, which might degrade myelin proteins. Since plasminogen is a major serum component, only a small amount of catalyst could produce a large amount of proteinase. Other macrophage products, such as lipases, other proteinases and oxygen radicals might also be involved. We suggested that the specificity of this mechanism for myelin might lie in the extreme sensitivity of myelin basic protein to proteolysis. Since the final effector cell is the macrophage this mechanism could be common to all forms of cell-mediated demyelination independent of antigen.

Five approaches were designed to explore this hypothesis: (1) the effects of macrophage depletion on EAE, (2) the reaction of macrophage secretion products on myelin *in vitro*, (3) the effects of complement plus proteinases on myelin *in vivo*, (4) the induction of demyelination *in vivo* with plasminogen activator, and (5) the suppression of EAE with proteinase inhibitors. Each of these approaches will be discussed briefly in turn.

1. Brosnan et al. (4) showed that EAE can be prevented if macrophages are selectively depleted by injecting silica dust intraperitoneally. All Lewis rats inoculated with guinea pig spinal cord in complete Freund's adjuvant develop severe clinical signs by days 14-15. Eighty percent of animals given i.p. injections of 200 mg of silica on days 8 and 11 failed to develop clinical signs for at least 4 weeks, and showed little inflammation and no demyelination. Lymphocytes taken from the silica-treated animals were, however, capable of passively transferring EAE to naive recipients.

These results strongly support the conclusion that macrophages function as the effector cells in the clinical and pathological expression of EAE. Thus, while the sensitized lymphocyte is the necessary immunocompetent cell for the initiation of the CMI response, the activated macrophage actually mediates the tissue damage.

2. We showed that macrophage secretion products could degrade the basic protein in lyophilized myelin *in vitro* and that this degradation could be considerably enhanced by the addition of plasminogen (10). These results suggested that plasmin was effective in hydrolyzing basic protein. To verify it we showed that urokinase, a known plasminogen activator, together with purified plasminogen, could degrade basic protein in myelin. These data showed that myelin is susceptible to degradation by products of activated macrophages.

3. Although macrophage secretion products alone, or together with plasminogen, could degrade myelin proteins in lyophilized myelin, they were ineffective when either freshly isolated myelin or fresh white matter was used as a substrate. We reasoned that some additional factor was needed to disrupt the myelin structure making it accessible to the proteinases. Two possible candidates were complement and phospholipases. Both were known to be secreted by macrophages under appropriate conditions, and of course serum complement would also be available in the lesion (reviewed in 9). Myelin is known to be able to activate complement in the absence of antibody or immune complexes (11, 32), and this activation, by means of the classical pathway, results in the incorporation of the pore-forming membrane-attack complex into the myelin (20, 27).

We found that pretreatment of fresh myelin with complement potentiated the degradation of basic protein by plasmin, or by macrophage conditioned media plus plasminogen. Complement depleted (heated) sera, C₃-deficient sera or C₄-deficient sera were ineffective (9). These data indicated that complement, activated by the classical pathway, could render the basic protein in fresh myelin vulnerable to proteolytic enzymes. Phospholipase or lysolecithin were also effective in potentiating the action of proteinases of fresh myelin.

4. If our central hypothesis, that proteinases initiate demyelination, is correct then plasmin itself should be able to cause demyelination in the absence of a CMI reaction. We tested this possibility in the rabbit eye, which is unique in that it has a strip of myelinated fibers lying on the surface of the retina exposed to the vitreous humor. Urokinase, a plasminogen activator, was injected into the posterior chamber of the eye (5). Demyelination was consistently observed in the superficial layers of the retina. A mononuclear cell infiltrate was also present, and demy-

lination was observed in the vicinity of the invading cells. Urokinase inactivated by diisopropylfluorophosphate was not effective in causing demyelination or inflammation.

5. If neutral proteinases have a key role in initiating the clinical signs and pathology of the inflammatory demyelinating diseases it might be expected that inhibitors of these enzymes would protect sensitized animals against EAE. EAE was induced in male Lewis rats. Starting on day 6 proteinase inhibitors were injected intraperitoneally twice daily, and sensitized control animals were injected with saline.

We found that aminomethylcyclohexane carboxylic acid (AMCA), ϵ -amino-caproic acid (EACA), and p-nitrophenylguanidinobenzoate (NPGB), all of which are inhibitors of plasminogen, plasmin and other neutral proteinases, gave significant protection against the clinical expression of EAE (6). Pepstatin, an inhibitor of acid proteinases, was also effective in protecting some of the animals. Leupeptin and antipain were ineffective at the dose used. Trasylol (aprotinin) actually exacerbated the disease. AMCA and NPGB were most effective in decreasing the extent of weight loss. Histological examination of clinically well animals treated with NPGB and pepstatin showed that both perivascular infiltration and submeningeal inflammation were markedly reduced. In asymptomatic animals treated with AMCA and EACA perivascular infiltration was reduced only slightly; however in AMCA-treated animals, the degree of demyelination in the vicinity of the inflammatory cells was reduced considerably.

Smith and Amaducci (28) carried out an extensive independent study of EAE protection using the same drugs we have tested. Their results are essentially in agreement with ours, including the finding that Trasylol exacerbates EAE.

All of the experimental work described here strongly supports our original postulate. These data show that macrophages are necessary for the development of EAE, an inflammatory demyelinating disease known to involve CMI reactions; that macrophage secretion products, especially in the presence of plasminogen and complement, are capable of degrading myelin proteins; that demyelination can be induced by a plasminogen activator; and that EAE can be suppressed by inhibitors of plasminogen activator and plasmin.

BLOOD-BRAIN BARRIER, EDEMA AND INFLAMMATION

Our hypothesis outlined above depends upon increased vascular permeability, permitting leakage into the brain parenchyma of the serum proteins, plasminogen and complement. It is known that alteration in

blood-brain barrier (BBB) permeability is an early and significant event in EAE. Several studies have shown that clinical signs of disease correlate more closely with the extent of edema in the spinal cord than with histological evidence of inflammation (16, 18), or with the degree of demyelination (21, 26). In fact, there is little or no demyelination in animals in which EAE is induced with pure myelin basic protein (MBP), although the clinical signs are as pronounced as in EAE animals induced with whole white matter, where demyelination is obvious (26). The factors involved in this alteration of BBB function are not well understood. In both the mouse model and the reactivated rat model antagonists of histamine can suppress clinical signs of disease, thus implicating this vasoactive amine in the disease process (19, 23). A possible requirement for vasoactive amines in the development of a delayed-type hypersensitivity (DTH) reaction has also been proposed (13). In DTH reactions the augmenting inflammatory response is known to play a major role in the initiation of tissue damage. This response consists primarily of cells of the monocyte macrophage lineage that do not normally leave the blood. Gershon et al. (13) have suggested that vasoactive amines cause constriction of the endothelial cells thus facilitating egress of bone marrow derived cells from the circulation.

If this hypothesis is correct then one would predict that only the specifically sensitized T-cell would elicit lymphokine production that results in vasoconstriction, edema, and a non-specific augmentation of the immune response. This hypothesis also involves the concept that all types of activated T-cells cross the endothelium into the parenchyma, and there is preliminary evidence from work with the T-cell lines that this is indeed the case. Thus, there would be two levels at which the response could be blocked: the receptor that mediates perivascular transit of activated T-cells, and the action of lymphokines on the vascular endothelium. Our work has focussed on the vascular response.

Preliminary studies indicated that antagonists of histamine and serotonin could not significantly suppress the development of clinical signs of EAE in the Lewis rat, and therefore we turned our attention to the catecholamines.

In most of the vasculature norepinephrine and epinephrine mediate vasoconstriction via the α -receptor and vasodilation via the β -receptor. We therefore tested the ability of antagonists of α and β adrenergic receptors to modulate the expression of EAE in the Lewis rat. Our results showed that significant suppression of the clinical and histological expression of EAE in the Lewis rat could be obtained by treatment with prazosin, a specific antagonist of α_1 -adrenoceptors (7). Treatment with either the α_2 -antagonist yohimbine or the β -antagonist-propranolol ex-

cerbated the disease, whereas treatment with the mixed α_1/α_2 -antagonist phenoxybenzamine had some suppressive activity. Treatment with prazosin was also able to suppress clinical and histological signs of EAE in animals sensitized by adoptive transfer with activated spleen or lymph node cells. Since the presynaptic α_2 -adrenoceptor exerts a negative feedback control on the release of norepinephrine, antagonism of this receptor could lead to unrestrained release of NE, which could account for the exacerbation of the disease observed in animals treated with yohimbine.

Further studies designed to examine the effect of prazosin on vascular permeability in EAE have shown that in both actively induced and passively transferred disease, treatment with prazosin significantly suppresses leakage of serum proteins into the spinal cord and delays the expression of the inflammatory response (15).

In order to explore the mechanism of action of prazosin further we have also tested the effect of prazosin on the early, inductive phase and on the late, effector phase of the disease (8). The results of these studies have shown that treatment with prazosin has no effect on the early, inductive phase of EAE but can still significantly suppress disease when treatment is begun at the time of onset of early clinical signs (day 10). Leakage of serum proteins and perivascular inflammation were also suppressed in these animals, particularly in the early stages of the acute response. Lymphocytes, obtained from both treated animals and from sensitized animals incubated in the presence of prazosin *in vitro*, showed that prazosin had no effect on lymphocyte responses to antigen or mitogen.

We have examined the astrocyte reaction as another indicator of disease. Smith et al. (30) have shown that in Lewis rats, sensitized to develop EAE, enhanced immunostaining for glial fibrillary acidic protein (GFAP) is evident early in the disease (10-12 days post-inoculation). The staining intensity increases with time and the reactive astrocytes are found throughout the spinal cord, unrelated to sites of inflammation. We confirmed these results and showed that this enhanced immunostaining for GFAP was delayed in rats in which the clinical signs of EAE had been suppressed by prazosin treatment (14).

To summarize the prazosin studies: we find that prazosin treatment suppresses clinical signs and edema, only partially suppresses and delays perivascular inflammation and delays the astrocyte response. Clinical signs, therefore, correlate well with increased vascular permeability and not with inflammation, whereas the onset of the astrocyte response correlates well with the onset of inflammation. It is also clear that enhanced GFAP staining does not correlate with clinical signs, since the glial reaction is still intense at 65 dpi (3), long after animals have recovered,

and enhanced staining occurs in prazosin-treated animals in the absence of clinical signs.

Prazosin is a common antihypertensive drug. Its activity is the result of vasodilation, believed to be caused by blockade of vascular α_1 -adrenergic receptors. Our results support the hypothesis that prazosin suppresses EAE through a direct vascular effect. This drug also allows us to distinguish the development and effects of inflammation from those of vascular permeability and provides a tool with which to explore the factors involved in edema.

CONCLUSION

The studies reviewed here concern several aspects of the induction and development of the cell-mediated immune lesion in EAE — from the opening of the BBB to the molecular mechanisms that may be responsible for demyelination. Although we recognize that EAE is an imperfect model of multiple sclerosis, the similarity in the pathology of the lesions in these two conditions justifies the use of this model disease to investigate what we believe may be universal mechanisms of inflammatory demyelination.

From our work and that of others, the following sequence of events can be proposed. Sensitized T-cells either invade the CNS and react with antigen (myelin basic protein in the case of EAE) presented by microglia or astrocytes, or react with antigen presented by the endothelial cell. Lymphokines are released which amplify the response by "calling in" monocytes and other lymphocytes. Cellular products from one or more of these inflammatory cell types induce the release of vasoactive amines which cause vasospasm leading to an increase in vascular permeability. Our evidence indicates that in EAE vasospasm is maintained by agonists acting on α_1 -adrenergic receptors. The resultant breakdown in the blood-brain barrier leads to vasogenic edema and increased perivascular transit of inflammatory cells. We have some evidence from our studies of passively-transferred disease that the increase of edema slightly precedes the increase in cellular infiltration.

The effector stage of disease, discussed above, leads subsequently to the augmented inflammatory response and a greatly increased infiltration of inflammatory cells. We believe that the activated macrophages are largely responsible for the tissue damage (demyelination) in the lesion, which is initiated by secretion products of these cells.

We have shown that this sequence of events can be interfered with at several stages, leading to suppression of various manifestations of

the disease. For example, blockade of the α_1 -receptor by prazosin during the inductive phase has no effect, suggesting that it has no effect on the immune response. However, blockade during the effector stage suppresses edema and clinical signs, but only delays inflammation and the astrocyte response. We are thus able to show that clinical disease correlates better with edema than with inflammation, whereas the astrocyte response is related to inflammation. We have also shown previously that clinical signs can be suppressed by depletion of macrophages and by treatment with proteinase inhibitors.

It is our hope that detailed studies of the pathogenesis of the model disease, EAE, will lead to logical ways of interfering with the progression of multiple sclerosis.

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REFERENCES

1. ADAMS, C. W. M. 1977. Pathology of multiple sclerosis: Progress of the lesion. *Br. Med. Bull.* 33: 15-20.
2. ADAMS, R. D. 1959. A comparison of the morphology of the human demyelination disease and experimental "allergic" encephalomyelitis. *In* M. W. Kies and E. C. Alvord (ed.), *Allergic encephalomyelitis*. Charles C. Thomas, Springfield, Illinois, 183 p.
3. AQUINO, D. A., CHIU, F.-C., BROSNAN, C. F. and NORTON, W. T. 1988. Glial fibrillary acidic protein increases in the spinal cord of Lewis rats with acute experimental autoimmune encephalomyelitis. *J. Neurochem.* 51: 1085-1096.
4. BROSNAN, C. F., BORNSTEIN, M. B. and BLOOM, B. R. 1981. The effects of macrophage depletion on the clinical and pathologic expression of EAE. *J. Immunol.* 126: 614-620.
5. BROSNAN, C. F., CAMMER, W., BLOOM, B. R. and NORTON, W. T. 1980. Initiation of primary demyelination *in vivo* by a plasminogen activator (urokinase). *J. Neuropathol. Exp. Neurol.* 39: 344.
6. BROSNAN, C. F., CAMMER, W., NORTON, W. T. and BLOOM, B. R. 1980. Proteinase inhibitors suppress the development of experimental allergic encephalomyelitis. *Nature* 285: 235-237.
7. BROSNAN, C. F., GOLDMUNTZ, E. A., CAMMER, W., FACTOR, S. M., BLOOM, B. R. and NORTON, W. T. 1985. Prazosin, an α_1 -adrenergic receptor antagonist, suppresses experimental autoimmune encephalomyelitis in the Lewis rat. *Proc. Natl. Acad. Sci. USA* 82: 5915-5919.
8. BROSNAN, C. F., SACKS, H. J., GOLDSCHMIDT, R. C., GOLDMUNTZ, E. A. and NORTON, W. T. 1986. Prazosin treatment during the effector stage of disease suppresses experimental autoimmune encephalomyelitis in the Lewis rat. *J. Immunol.* 137: 3451-3456.

9. CAMMER, W., BROSNAN, C. F., BASILE, C., BLOOM, B. R. and NORTON, W. T. 1986. Complement potentiates the degradation of myelin proteins by plasmin: Implications for a mechanism of inflammatory demyelination. *Brain Res.* 364: 91-101.
10. CAMMER, W., BLOOM, B. R., NORTON, W. T. and GORDON, S. 1978. Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: A possible mechanism of inflammatory demyelination. *Proc. Natl. Acad. Sci. USA* 75: 1554-1558.
11. CYONG, C.-J., WITKIN, S. S., RIEGER, B., BARBARESE, E., GOOD, R. A. and DAY, N. K. 1982. Antibody-independent complement activation by myelin via the classical complement pathway. *J. Exp. Med.* 155: 587-598.
12. DAL CANTO, M. C., WISNIEWSKI, H. M., JOHNSON, A. B., BROSTOFF, S. W. and RAINE, C. S. 1975. Vesicular disruption of myelin in autoimmune demyelination. *J. Neurol. Sci.* 24: 313-319.
13. GERSHON, R. K., ASKENASE, P. W. and GERSHON, M. D. 1975. Requirement for vasoactive amines for production of delayed-type hypersensitivity skin reactions. *J. Exp. Med.* 142: 732-747.
14. GOLDMUNTZ, E. A., BROSNAN, C. F., CHIU, F.-C. and NORTON, W. T. 1986. Astrocyte reactivity and intermediate filament metabolism in experimental autoimmune encephalomyelitis: The effect of suppression with prazosin. *Brain Res.* 397: 16-26.
15. GOLDMUNTZ, E. A., BROSNAN, C. F. and NORTON, W. T. 1986. Prazosin treatment suppresses increased vascular permeability in both acute and passively transferred experimental autoimmune encephalomyelitis in the Lewis rat. *J. Immunol.* 137: 3444-3450.
16. KERLERO de ROSBO, N., BERNARD, C. C. A., SIMONS, R. D. and CARNEGIE, P. R. 1985. Concomitant detection of changes in myelin basic protein and permeability of blood-spinal cord barrier in acute experimental autoimmune encephalomyelitis by electroimmunoblotting. *J. Neuroimmunol.* 9: 349-361.
17. LAMPERT, P. W. 1965. Demyelination and remyelination in experimental allergic encephalomyelitis: Further electron microscopic observations. *J. Neuropathol. Exp. Neurol.* 24: 371-385.
18. LEIBOWITZ, S. and KENNEDY, L. 1972. Cerebral vascular permeability and cellular infiltration in experimental allergic encephalomyelitis. *Neurology* 22: 859-869.
19. LINTHICUM, D. S. and FRELINGER, J. A. 1982. Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. *J. Exp. Med.* 155: 31-40.
20. LIU, W. T., VANGURI, P. and SHIN, M. L. 1983. Studies on demyelination in vitro: The requirement of membrane attack components of the complement system. *J. Immunol.* 131: 778-782.
21. MOORE, G. R. W., TRAUGOTT, U., FAROOQ, M., NORTON, W. T. and RAINE, C. S. 1984. Experimental autoimmune encephalomyelitis: Augmentation of demyelination by different myelin lipids. *Lab. Invest.* 51: 416-424.
22. NORTON, W. T. and CAMMER, W. 1984. Chemical pathology of diseases involving myelin. In P. Morell, (ed.), *Myelin*. 2nd ed. Plenum Press, New York, 369 p.
23. PRINEAS, J. W., KWON, E. E., CHO, E.-S. and SHARER, L. R. 1984. Conti-

- nual breakdown and regeneration of myelin in progressive multiple sclerosis plaques. *Ann. N.Y. Acad. Sci.* 436: 11-32.
24. RAINE, C. S. 1983. Multiple sclerosis and chronic relapsing EAE: Comparative ultrastructural neuropathology. *In* J. F. Hallpike, C. W. M. Adams, and W. W. Tourtellotte (ed.), *Multiple sclerosis, pathology, diagnosis and management*. Chapman and Hall, London, p. 413.
 25. RAINE, C. S., SNYDER, D. H., VALSAMIS, M. P. and STONE, S. H. 1974. Chronic experimental allergic encephalomyelitis in inbred guinea pigs: An ultrastructural study. *Lab. Invest.* 31: 369-380.
 26. RAINE, C. S., TRAUOGOTT, U., FAROOQ, M., BORNSTEIN, M. B. and NORTON, W. T. 1981. Augmentation of immune-mediated demyelination by lipid haptens. *Lab. Invest.* 45: 174-182.
 27. SILVERMAN, B. A., CARNEY, D. F., JOHNSTON, C. A., VANGURI, P. and SHIN, M. L. 1984. Isolation of membrane attack complex of complement from myelin membranes treated with serum complement. *J. Neurochem.* 42: 1024-1029.
 28. SMITH, M. E. and AMADUCCI, L. A. 1982. Observations on the effect of protease inhibitors on the suppression of experimental allergic encephalomyelitis. *Neurochem. Res.* 7: 541-554.
 29. SMITH, M. E. and BENJAMINS, J. A. 1984. Model systems for study of perturbations of myelin metabolism. *In* P. Morell (ed.), *Myelin*. 2nd ed. Plenum Press, New York, p. 441.
 30. SMITH, M. E., SOMERA, F. P. and ENG, L. F. 1983. Immunocytochemical staining for glial fibrillary acidic protein and the metabolism of cytoskeletal proteins in experimental allergic encephalomyelitis. *Brain Res.* 264: 241-253.
 31. UNKELESS, J. C., GORDON, S. and REICH, E. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* 139: 834-850.
 32. VANGURI, P., KOSKI, C. L., SILVERMAN, B. and SHIN, M. L. 1982. Complement activation by isolated myelin: Activation of the classical pathway in the absence of myelin-specific antibodies. *Proc. Natl. Acad. Sci. USA* 79: 3290-3294.
 33. WAXMAN, F. J., BERGMAN, R. K. and MUNOZ, J. J. 1982. Abrogation of resistance to the reinduction of experimental allergic encephalomyelitis by pertussigen. *Cell Immunol.* 72: 375-383.

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