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Arteriosclerosis: Is Stress-Induced Immune Suppression a Risk Factor?

Abstract. *Female Sprague-Dawley rats, purchased as retired breeders, developed arteriosclerosis that was accompanied by immune complex deposition in the arterial lesion and depressed immune responsiveness to T cell mitogens.*

Repeatedly bred female Sprague-Dawley rats develop a spontaneous arteriosclerotic condition characterized by medial necrosis and calcification, endothelial proliferation, thymus involution, and accelerated aging (*1*). These lesions, similar to those observed in humans, occur in 80 to 90 percent of the rats that have produced three or more litters (approximately ten pups per litter). The lesions begin in the lower abdominal aorta and are found throughout the body in many instances. Although hormones of the hypothalamic-pituitary-adrenal axis, diet, and other factors have been implicated in the pathogenesis of this disorder (*2*), an involvement of the immune system is also a possibility. Other researchers have investigated stress-induced alteration of the immune system (*3*), but this condition has not been linked to arteriosclerosis.

The animals used in these experiments were female Sprague-Dawley rats, purchased as retired breeders (*4*). Female virgin rats of similar ages, the same strain, and from the same supplier were used as controls. All the rats were fed standard rat chow prepared by ARS Sprague-Dawley laboratories prior to purchase, and in our laboratory they were fed Purina Rat Chow. The animals were given unrestricted access to food and water.

The lesions that developed in these rats appeared to be similar to those seen in serum sickness (*5*) and immune complex disorders (*6*). To characterize these lesions, we prepared frozen sections of aortas from diseased as well as control animals. The sections were stained with peroxidase-labeled rabbit antibody to rat immunoglobulin G (IgG) (*4*, *7*). This procedure demonstrated significant quantities of IgG in the medial layer of the vessel affected with the lesion (Fig. 1); no such deposits appeared in the controls. To determine if the IgG was directed against the constituents of the diseased vessel wall we conducted a hemagglutination assay with glutaraldehyde-fixed sheep red bloods cells (RBC) to which the antigen was covalently bound with *N*-ethyl-*N'*-(dimethylaminopropyl)-carbodiimide hydrochloride. Double diffusion tests in agar were also performed with a homogenized extract of diseased aorta being used as the antigen. Titers of the hemagglutination tests were the same for the serum from the diseased and control animals. All results of the diffusion tests were negative. These findings indicate that the antibody visible in the lesion was not directed against the arterial wall.

The IgG in the medial lesion probably represents deposits of an antigen-antibody complex similar to the immune complex deposits found in various tissues in a number of human conditions of

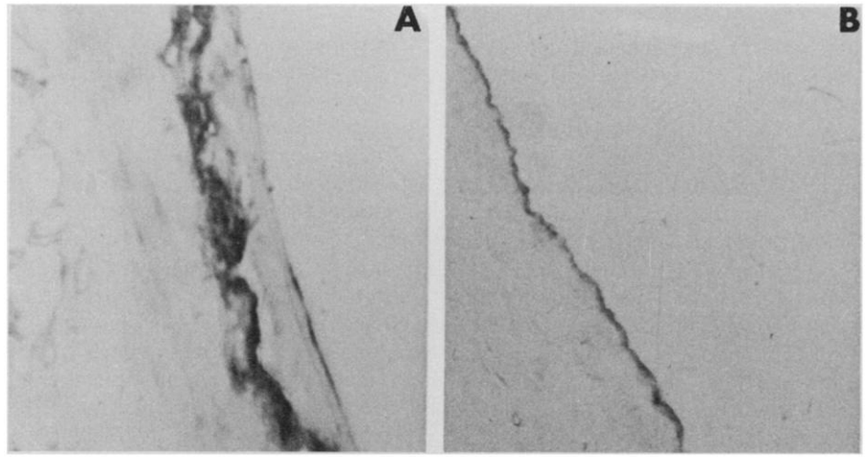

Fig. 1. (A) Immunoglobin G deposits in the medial layer of the abdominal aorta from the arteriosclerotic rat. (B) Normal control animal. (×160)

Table 1. Comparison of the response of spleen cells from arteriosclerotic and control animals to the mitogens PHA, Con A, and LPS, and the response of the spleen cells in the direct plaque-forming cell assay. Abbreviation: Amt., amount.

Amt. (μl/well)	Mitogens (count/min × 10^{-3})								Plaque-forming cells (plaques per 10^6 spleen cells)	
	PHA		Con A			LPS				
	Arteriosclerotic	Control	Amt. (μg/well)	Arteriosclerotic	Control	Amt. (μg/well)	Arteriosclerotic	Control	Arteriosclerotic	Control
0	6.9 ± 1.3	6.6 ± 0.9	0	4.3 ± 1.6	3.8 ± 0.8	0	13.5 ± 1.0	10.1 ± 0.5	219 ± 55	229 ± 67
2	13.6 ± 6.0	22.1 ± 2.4	0.5	8.2 ± 0.9*	74.2 ± 8.9	2	23.8 ± 1.1	17.1 ± 0.5		
4	13.5 ± 2.7*	34.5 ± 3.8	1	12.5 ± 1.3*	77.5 ± 6.1	4	25.0 ± 1.7	18.6 ± 1.0		
6	12.3 ± 2.5*	39.4 ± 3.9	2	12.3 ± 1.6*	59.5 ± 16.7	6	23.3 ± 1.1	16.0 ± 0.2		

*$P < .005$.

unknown etiology as well as in the well-characterized diseases such as systemic lupus erythematosus (*6*) and multiple sclerosis (*8*). In such conditions varying degrees of immune suppression or exhaustion are also demonstrated (*9*). With this in mind, the spleen cell responses to the T cell mitogens phytohemagglutinin M (PHA) and concanavalin A (Con A) and the B cell mitogen *Escherichia coli* lipopolysaccharide (LPS) (*4*) were examined (*10*). All mitogen experiments were performed in triplicate, three to six animals being used per experiment. With PHA, although the degree of responsiveness varied with the animal, the mean response of the arteriosclerotic animals was depressed as much as 400 percent from that of the controls. Similar results were obtained with Con A. No significant differences were noted with LPS (Table 1).

The mixed lymphocyte reaction, an indication of T cell responsiveness, was used to further characterize T cell reactivity (*11*). A mixed lymphocyte reaction would be expected to occur because Sprague-Dawley rats are not totally syngeneic. When the spleen cells of three arteriosclerotic animals were pooled, there was no increase in [^{3}H]thymidine uptake compared with the uptake in separate cultures. An approximate twofold increase in [^{3}H]thymidine uptake occurred in the pooled cells of three control animals. These findings give further indication of a depressed T cell function in the arteriosclerotic animal.

To verify normal B cell function in the LPS studies above, we assessed the antibody response of the arteriosclerotic animals to sheep RBC using the direct plaque-forming cell technique (*12*). Both arteriosclerotic and control animals were sensitized with 0.5 ml of a 10 percent suspension of sheep RBC and the number of plaques was counted on day 5. There was no significant difference between the mean response of the two groups of animals (Table 1). These results indicate that the T cell–dependent antibody response of the diseased animal was not altered. Since no decrease in activity was noted among the population of T cells involved in the antibody response to sheep RBC, whereas T cells involved in the mixed lymphocyte reaction and in the mitogen response were significantly suppressed, this indicates that different populations of T cells were involved.

The arteriosclerotic animals manifested significant T cell depression. It may be postulated that the suppression is due to multiple pregnancies that increase the amount of suppressor substances (*13*); however, the stress, which is known to increase cortisone production (*3*), may also deplete the spleen of functional T cells. Depression of T cells has been demonstrated in aging mice (*14*), autoimmune disease (*9*), virus infections (*15*), and in some experimental tumor models (*11*). The antigen-antibody complexes demonstrated in the vessel wall cannot be attributed to an autoimmune phenomenon to arterial tissue, but we advance the hypothesis that these complexes might include viral, environmental, or other tissue antigens, since similar deposits have been demonstrated in other models (*16*).

We suggest that this model of arteriosclerosis might be included in the same category of disorders as some autoimmune or viral conditions that manifest immune complex deposition in tissues as well as significant immune suppression. However, it should be emphasized that our data demonstrate that the stress of multiple pregnancies results in immune suppression.

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11. For the mixed lymphocyte reaction, spleens from three animals were combined, and 0.1 ml (6×10^6 viable nucleated spleen cells) was added to the wells of a microculture plate. Medium (0.1 ml) was added to each well and the culture was incubated at 37°C in 5 percent CO_2. After 24 hours the cultures were exposed to 0.5 μc of [^{3}H]thymidine. The cultures were then incubated and harvested as in (*10*).
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Paternity and Genetic Heterogeneity in the Polygynous Bat, *Phyllostomus hastatus*

Abstract. *Wild colonies of greater spearnose bats were marked, censused regularly, and genotyped at three polymorphic allozyme loci. While adult composition of social units is very stable and strong polygyny results in marked changes in gene frequencies between generations, dispersal of offspring is sufficient to prevent significant genetic heterogeneities between social units. Kin selection cannot explain social cohesiveness in these highly social mammals.*

Social organization in animal populations can reduce genetically effective population sizes and restrict gene flow between adjacent social units. These effects and their evolutionary consequences are maximized when three features act in concert: (i) a low exchange rate of adults between groups; (ii) a preferential recruitment of juveniles into their parental groups; and (iii) a restriction of mate selection to members of the same social unit, coupled with a large disparity in mating success among members of one sex. Recent treatments support the argument that the combined effects of these features are to generate substantial genetic heterogeneity among social units, result in rapid fixation of initially rare genetic characters, and perhaps lead to reproductive isolation and speciation (*1*). Since these latter effects are all important stages in evolutionary processes, it seems particularly important to know (i) how frequently the three social features are acting in concert and (ii) how easily a dissonant feature can counter the effects produced by two sympathetic features.