Possible Mechanisms of Vasectomy-exacerbated Atherosclerosis*

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Abstract
Our laboratory as well as those of others have demonstrated that in experimental animals vasectomy results in immune-complex deposition not only in the reproductive tract but also in the renal glomerulus. We have shown that in two species of monkeys vasectomy results in a significant increase in atherosclerosis and have postulated that this may be due to circulating immune complexes. We have shown a mild change in arteriolar vessels in a small study of vasectomized men and have found a mild but insignificant increase in systolic blood pressure in vasectomized men over time compared to an age-matched group. One cannot ignore the fact that persistent autoimmune responses to spermatozoal antigens are generated in both vasectomized men and animals. The paucity of direct information about whether vasectomy exacerbates atherosclerosis in human subjects has made reliance on animal studies unavoidable. But to date there is no evidence that vasectomy causes a similar effect in human beings.

Introduction
The prototype of immune-complex disease is considered to be serum sickness. This disease, first described in man by von Pirquet and Schick in 1905, has been studied extensively in experimental animals. Exposure to a large quantity of heterologous protein can cause the formation of antibodies; when the antigen and antibody combine, immune complexes result. Such complexes play important roles in the pathogenesis of many disorders, including infections, autoimmune diseases, and malignant diseases. The deposition of immune complexes is widely accepted as the major process mediating immunologic renal disease in humans (Dixon and Wilson 1978).

Immune-Complex Disease
Experimental immune-complex disease has been studied most extensively in rabbits. Both acute (one-shot) and chronic (multiple-injection) approaches have been used. Use of a single injection of a foreign protein such as bovine serum albumin (BSA) or bovine gamma globulin (BGG) allows evaluation of the relationship between the fate of the injected antigen, the appearance of the antibody, and the subsequent development of disease. Researchers have repeatedly demonstrated that no symptom of disease (neutropenia, enlarged lymph nodes, glomerulonephritis, or arteritis) occurs until the appearance of soluble immune complexes in the circulation. At this point

* Publication No. 1204 of the Oregon Regional Primate Research Center. This paper was presented as the 1981 James Goding Memorial Lecture at the Annual Meeting of the Australian Society for Reproductive Biology, Christchurch, New Zealand, in August 1981.
the complement cascade becomes activated by an exposed portion of the antibody molecule known as the Fc region. This results in a decrease in serum complement levels. As antibody production continues, the ratio of the concentration of antibody to antigen increases, larger immune complexes are formed, and the antigen is eliminated via the reticuloendothelial system. The course of the disease is limited to the interval in which immune complexes that may affect the arteries, glomeruli, joints, and pericardium are present in the circulatory system.

Immunologically mediated injury is thought to occur because of deposition of preformed complexes in either the renal glomerulus or the blood vessel wall. This hypothesis is based on the interval between injection of the foreign antigen and evidence of disease. Injection of preformed soluble immune complexes results in vascular damage as early as 36 h later. This fact strongly indicates that an immune response (i.e. antibody production) need not be mounted for damage to occur (McCluskey et al. 1962). When complexes have been infused into normal animals, arteritis, endocarditis, and glomerulonephritis have developed (McCluskey et al. 1962). However, glomerular localization of these preformed immune complexes by means of immunofluorescence (a common method to evaluate localization) has not been demonstrated, possibly because of the dose of the infused immune complexes. Similarly, subendothelial deposits like those found in chronic serum sickness are usually not produced by preformed immune complex infusions.

Characteristics of Complexes

The formation and biological activities of circulating immune complexes depend upon the type of antibody and antigen. The valence, size, and charge of both antigen and antibody determine whether the complexes result in a detrimental effect. Complexes formed in moderate antigen excess, that is, those consisting of at least two IgG antibody molecules, are small enough to be soluble and thus remain in the circulation but are large enough to activate complement. The class and subclass of the antibodies determine their valency as well as their ability to bind complement. The IgG subclasses 1, 2, and 3 can interact effectively with complement and thus may be more damaging than IgG 4, which binds weakly.

Complement activation is not the only mechanism of action of immune complexes. Many cells can respond directly to these complexes by means of receptors for Fc on their surfaces. Fc receptors for IgG have been recognized on cells of the lymphoid line as well as on monocytes, neutrophils, eosinophils, platelets, and endothelial cells. Human neutrophils, for example, are bound to aggregated IgG of all four subclasses. Macrophage receptors, on the other hand, may be limited to subclasses IgG 1 and 3.

Immune-complex Clearance

Clearance of immune complexes depends upon the degree of lattice formation, that is, the molar ratio of antigen to antibody. Large lattices formed in antibody excess are rapidly removed from the blood stream by the reticuloendothelial system, primarily the Kupffer cells of the liver (Fig. 1). Complexes formed in great antigen excess will be of a small size and may remain in the circulation but have weak phlogistic potential. Of particular immunogenic importance are immune complexes formed in moderate antigen excess because they may remain in the circulation, become widely disseminated, and activate the complement cascade.
Continuous secretion or release of antigenic material in chronic infections or in autoimmune disease processes may lead to enduring immune-complex formation, which may result in clinical manifestations. In glomerulonephritis the type of response depends upon the type of antibody that is formed. In individuals that produce copious amounts of antibody (good responders) large complexes develop, and these are rapidly eliminated from the circulation. Moderate responders have smaller, more soluble immune complexes that result in mesangial and subendothelial deposits. Poor antibody responders remain in persistent antigen excess and may exhibit only very small soluble immune complexes associated with subepithelial deposition.

![Fig. 1](image1.jpg)  
**Fig. 1.** Brightly stained Kupffer cells (arrowheads) in rabbit liver that have been incubated with a fluorescein-conjugated anti-IgG indicate the uptake of immune complexes in an animal that received chronic injections of bovine serum albumin. Complex size is important in Kupffer cell uptake. After vasectomy, no immune staining of such cells is found. Bar = 100 μm.

![Fig. 2](image2.jpg)  
**Fig. 2.** C3 [fluorescein isothiocyanate (FITC)-conjugated anti-C3] deposition (arrowheads) around the seminiferous tubules of a vasectomized rabbit indicate antigen leakage and tissue-associated complexes. Bar = 100 μm.

Other factors play important roles in the clearance mechanism. For example, the functional states of the host’s circulating and fixed phagocytes are important in determining the fate of the circulating immune complexes. If the reticuloendothelial system is impaired, removal of these complexes may be slowed. Genetic factors are also important. Studies of inbred mice suggest that an immune complex containing a low-affinity antibody and a weak lattice structure is removed more slowly from the circulation than a high-affinity antibody complex. The variability of autoimmune responses in human beings may stem from genetic differences.

**Tissue Deposition**

That immune complexes can be observed in tissues is direct evidence of their role in immunologic injury. Based on immunofluorescence evidence, immune deposits
that commonly occur in a discontinuous granular or lumpy-bumpy pattern are considered pathognomonic of immune-complex deposition (Couser and Salant 1980). Biopsies of affected tissue are an important tool for the detection of immune complexes because the presence of antibody, complement, and sometimes antigen may be determined or measured. The simultaneous presence of antigen or antibody plus complement strongly suggests immune-complex deposition (Wilson and Dixon 1974). If enough tissue is available, deposits can be eluted by the use of low pH buffers or chaotrophic agents. After such treatments, antibodies and occasionally antigens can be recovered and identified. Further definition of immune complexes may be accomplished by electron microscopy. The complexes appear as electron densities in the basal lamina.

Circulating immune complexes may become localized in vessel walls via several routes: (a) the complexes may be removed from the circulation by endothelial cells; (b) the complexes may tend to adhere to and become lodged in vessel walls, perhaps because of sticky endothelial cells; and (c) the complexes may be trapped by a filtering structure such as the glomerulus, which is in a state of increased permeability and allows the immune complexes to penetrate the wall of the vessel.

Vasectomy

Cutting and tying of the vasa deferentia blocks these excurrent ducts. In many species spermatogenesis continues relatively unabated, and sperm accumulate in the epididymis. Antibodies against sperm antigens form after vasectomy. Most likely these sperm antigens become exposed to the immune system via leakage of soluble products from the epididymis or testis. Immunoglobulins and complement have been found around the seminiferous tubules of vasectomized rabbits (Fig. 2) and around the excurrent duct system of vasectomized monkeys. Such findings pinpoint the site(s) of antigen leakage after vasectomy.

Spermatozoa produced after vasectomy are a constant source of antigen, and result in antibody production. The presence of antigen and antibody could be expected to mirror immune-complex disease. If vasectomy and immune-complex disease are similar, one would expect to find some evidences of glomerular deposition or of vascular changes in vasectomized animals and humans.

Glomerulonephritis

The glomerulus is particularly vulnerable to immune complexes because as it filters it may be subjected to a higher pressure gradient. Macromolecules could be trapped within the glomerular basement membrane. In immune-complex-initiated glomerulonephritis, histological examination reveals little leukocytic infiltration. By electron microscopy, swelling of the endothelial cells and thickening of the capillary walls are evident. Macrophages attracted by chemotactic substances may play an important role in the pathogenesis of the lesions (Hunsicker et al. 1979; Holdsworth et al. 1980).

In experimental immune-complex disease, if the quantity of antigen each day is in excess of the antibody production, chronic glomerulonephritis results. However, the severity may vary because antibodies of different classes, subclasses, and avidities may develop in different rabbits. Immunofluorescence can be used to determine the location of the complexes. In experimental serum sickness, both the antigen and the
Fig. 3. Large granular IgG deposits (arrowheads) (FITC-conjugated anti-IgG) in a renal glomerulus of a rabbit repeatedly immunized with BSA. Bar = 100 μm.

Fig. 4. A renal glomerulus of the same rabbit as in Fig. 3 stained to reveal BSA (FITC-conjugated anti-BSA). The presence of both immunoglobulin and antigen more surely indicates immune complex deposition. Bar = 100 μm.

Fig. 5. Renal glomerulus from vasectomized rhesus monkey stained to reveal a granular deposition of IgG (arrowheads). Bar = 100 μm.

Fig. 6. Renal glomerulus from a vasectomized rhesus monkey stained to demonstrate C3 deposits. Such deposits were only found in the vasectomized group. Bar = 100 μm.
antibody are known and thus their location can be observed within the glomerulus (Figs 3 and 4).

Fig. 7. Aortic arch from a rabbit repeatedly immunized with BGG. When stained with fluorescein-conjugated anti-BGG, deposition (arrowheads) within the arterial media is visible. Bar = 100 μm.

Fig. 8. When the same section as Fig. 7 is stained to reveal albumin, none is apparent, suggestive of selective vascular permeability. Bar = 100 μm.

If vasectomy results in immune-complex disease, evidence of immune-complex deposition should be found within the glomerular basement membrane. Such deposition is found in a percentage of rabbits that exhibit glomerular changes similar to
those observed in experimental serum sickness after vasectomy. Increased mesangial matrix and cellular proliferation are seen. Immunofluorescence and electron microscopic observation have revealed immune-complex deposition (Bigazzi et al. 1976; Clarkson and Alexander 1979). In monkeys, deposition of both IgM and IgG in a granular pattern can be observed (Fig. 5). Furthermore, the most prevalent complement component, C3, was found to be deposited only in glomeruli of vasectomized animals (Fig. 6) (Alexander and Tung 1979).

**Arteritis**

Immune-complex-mediated arteritis is thought often to involve the interaction of the complexes with polymorphonuclear granulocytes (Wedmore and Williams 1981). Activated polymorphonuclear granulocytes release a variety of lytic agents that can degrade cartilage, elastin, and nucleic acids, and degradation may result in inflammation and arterial wall damage.

Immune complexes can affect platelets directly or indirectly via complement or the activation of other cells. Platelets—reservoirs of vasoactive amines—play an important role in immune-complex-mediated arteritis.

Arteritis occurs most commonly at coronary outflow areas and points of bifurcation, perhaps because of hydrodynamic forces. The first steps in the development of arteritis involve an initial proliferation of intimal endothelial cells plus an increase in vascular permeability. The immune-complex permeates the blood vessel wall. This vascular permeability is specific. Arteries from rabbits with immune-complex disease have immune complexes but no evidence of albumin localization (Figs 7 and 8).

**Vasectomy and Arteritis**

We have sought to determine whether there is any evidence of arteritis after vasectomy. Even in experimental animals, immune-complex-induced arteritis is a patchy occurrence, i.e. it will be found in different locations in different animals. Its study in man is very difficult. Because the eye is a unique chamber in which to evaluate vascular changes, we studied arteriolar constriction in the eyes of 159 men, 30% of whom had been vasectomized. Constriction, albeit mild, was more common in vasectomized men under 40 years of age than in a similar group of men who had not been vasectomized (Fahrenbach et al. 1980). Whether further studies and the use of photographic evaluation will validate this initial finding remains to be determined.

One might postulate that if atherosclerosis occurs there would be an arterial stiffening which could result in an increase in systolic blood pressure. A study in cynomolgus and rhesus macaques revealed that arterial pulse wave velocity (related to the square root of arterial stiffness) was increased with increasing atherosclerosis (Farrar et al. 1980). If vasectomy hastens the rate of atherogenesis in man it is conceivable that blood pressure, particularly systolic blood pressure, could be affected. When the effect of vasectomy on blood pressure was evaluated on 946 male volunteer blood donors, 30% of whom were vasectomized, it was noted that there was a rise, although it was very small and non-significant, in systolic blood pressure (Alexander et al. 1981). This finding is of sufficient interest to suggest that a sample of men who have been vasectomized for longer periods be evaluated to determine whether a significant rise may occur.
A useful technique to intensify and localize arteritis has been to exacerbate the arterial injury by increasing the circulating levels of serum cholesterol. Ross and Glomset (1976) suggest that atherogenesis is the result of recurrent damage to the arterial intima or endothelial lining. After the initial damage, platelet-stimulated migration and proliferation of smooth muscle cells from the media to the intima occur. In the presence of repeated endothelial damage, the smooth muscle cells become overwhelmed with the cholesterol-bearing lipoproteins (mainly low-density lipoproteins). This deposition results in cell necrosis and the accumulation of lipid-laden cells that are typical of atheromatous plaques. Continued arterial injury results in ulcerated ruptured plaques and the release of thrombi. During this whole process there is a significant narrowing of the lumina of the involved arteries. The number and size of atheromatous lesions are greater when the regimen comprises both a high-cholesterol diet and injections than when the regimen consists of either an atherogenic diet or injections of foreign protein. We have tried to determine whether vasectomy causes effects similar to those observed in immune-complex disease. In a preliminary study, we used 10 cynomolgus macaques (Macaca fascicularis) maintained on a diet containing 0·50 mg of cholesterol per Calorie for 6 months. We then divided them into equivalent groups on the basis of their plasma cholesterol levels. One group was vasectomized; the other monkeys underwent sham operations. After 10 months, the experiment was terminated and the animals were killed. Atherosclerosis was significantly more extensive in the vasectomized than in the sham-operated animals. Deposition of cholesterol and cholesterol ester was significantly greater in the carotid, abdominal, iliac, and femoral arteries of the vasectomized animals. Plaque deposition in the intercranial cerebral arteries, a phenomenon previously observed only in hypertensive monkeys, was also apparent (Alexander and Clarkson 1978).

In a second study performed on animals fed Purina Monkey Chow (15% protein; Ralston Purina Co., St. Louis, Missouri), which is devoid of cholesterol and low in fat, an increase in atherosclerosis in the vasectomized animals was also demonstrated. Even though both control and experimental animals were fed a normal diet and had low levels of plasma lipids, the vasectomized monkeys had more extensive and severe atherosclerosis (Clarkson and Alexander 1980).

**Vasectomy and Atherosclerosis in Man**

We have demonstrated that in rhesus and cynomolgus macaques vasectomy exacerbates the development of atherosclerosis. It is important, yet much more difficult, to determine whether a similar effect occurs in men. We know a great deal about the prevalence of atherosclerosis in human beings and potential risk factors from various epidemiologic studies.

The term risk factor has been widely used in studies on coronary heart disease (CHD). Measured levels of the factor can be used to predict CHD. Nevertheless, since causality can only be proved by intervention trials, cause and effect relationships have not been demonstrated for most of the suggested risk factors. A risk factor may be merely a concomitant variable rather than a cause.

At least 246 factors have been suggested to be associated with the exacerbation of CHD (Hopkins and Williams 1981), but two-thirds of all CHD can be attributed to hypertension, hypercholesterolemia, and cigarette smoking (Stamler 1978). Other
major factors in the development of CHD include diabetes, age, sex, and familial predisposition for early CHD.

Because risk factors are often highly interrelated, studies on the relative importance of each factor are difficult.

Several prospective clinical studies of vasectomized men have been completed or are under way. In some of the first studies, small numbers of patients ranging from 50 to 200 were evaluated for a variety of medical, endocrine, and immunological tests. In one study, admissions of vasectomized men were followed through Scottish hospital records for approximately 5 years, and no untoward effects that could be related to vasectomy were observed (Goldacre et al. 1979).

It is possible that the effects of vasectomy may be evident only after long periods of time. Atherosclerosis in monkeys was observed 9–14 years after vasectomy, and if a similar trend were true for men, a considerable time period would be required for any differences between a vasectomized and control group. Four major studies are under way to attempt to extend the current knowledge of the relationship between vasectomy and vascular disorders in men (Anon. 1980). Since it is difficult to study atherogenesis directly, these studies evaluate the gross increases in the incidence of myocardial infarction as one end-point. The clinical occurrence of myocardial infarction is only loosely associated with the extensiveness of coronary artery atherosclerosis. To date, there is no direct evidence that vasectomy results in an increase in myocardial infarctions in men. Of the entire population at risk for myocardial infarction, the subset of vasectomized males represents a very small proportion. There could be an increase in relative risk for coronary heart disease among a small subset of vasectomized subjects that would not cause an impact in an obvious way on the total population. It may be that a modest elevation in levels of circulating lipids combined with vasectomy may cause an exacerbation of atherosclerosis but scientists will have to study sufficiently large samples and evaluate the plasma lipid levels of vasectomized and non-vasectomized men in order to determine whether the effect occurs in men. The results of such thorough evaluations of the effect require sensitive, complicated, epidemiologic studies.

Immune Complexes and Disease

Recently the pathological role of immune complexes has been questioned. Circulating immune complexes have been found regularly in the absence of overt glomerular immune deposits. Furthermore, they have been found in a variety of immune disorders that are not generally associated with immune-complex glomerulonephritis (for example, cancer and liver disease). Recent data suggest that damage may be induced in situ with a subsequent initiation of inflammatory responses that culminate in vascular lesions. This idea is in contrast to the dogma that complexes form in circulation and are subsequently deposited. When concanavalin A (con A), a glycoprotein-binding lectin, was used to locate an antigen on the glomeruli of rats, binding resulted in proliferative glomerulonephritis. Immunoglobulin, con A, and complement component 3 (C3) were present in the affected kidney. This finding suggests that certain proteins may bind to sugar residues in either the vascular or glomerular basement membrane and when the host mounts an antibody response, the immune complexes form in situ and thus result in inflammation. Perhaps the glomerulus
and even the endothelium have unique properties that allow immune-complex deposition in situ. In classical one-shot serum sickness studies, glomerular deposits containing the same antigen present in the circulating immune complexes are not found until antibody production has occurred. With certain antigens, however, this situation may be changed. Such antigens may have a distinct affinity for either a glomerular or an arterial basement membrane. Antigen deposition may occur as a primary event.

In the case of vasectomy, sperm antigens may have unique properties and thus they may be preferentially bound to certain surfaces. For example, Koehler and associates (1980) have shown that living sperm have a strong affinity for collagen and will adhere to the filaments by their heads. Localization studies have shown that acrosomal antigens are involved in this phenomenon. In some cases vasectomy could have an effect far and beyond what would be expected solely because of circulating immune complexes.

Much remains to be done before the role of sperm antigens and antibodies after vasectomy is understood. The rabbit and monkey have proved to be interesting models for basic research on immune complexes, vasectomy, and its effects.

Acknowledgments

The work described in this article was supported in part by National Institutes of Health Grant RR-00163 and National Institutes of Health Biomedical Research Support Grant S07RR05694.

References


Manuscript received April 22 1982, accepted July 22 1982