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ARTICLE *in* GENETICA · FEBRUARY 1995

Impact Factor: 1.4 · DOI: 10.1007/BF01435006

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Genetica **95:** 133–156, 1995.

Semen alloantigens and lymphocytotoxic antibodies in AIDS and ICL

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Received 27 January 1994; Accepted 17 June 1994

Key words: AIDS, alloantigen, autoimmunity, lymphocytotoxic antibodies, semen

Abstract

More than 90% of people with AIDS develop circulating immune complexes (CICs) and lymphocytotoxic antibodies (LCTAs). Animals infected with HIV, however, never display CICs or LCTAs, and remain healthy. Similarly, HIV-infected people who do not develop CICs or LCTAs also do not progress to AIDS. The appearance of CICs and LCTAs is, however, highly prognostic for AIDS and death. Since HIV infection does not, *per se*, lead to the development of CICs and LCTAs, other causes are likely. One such cause, for which both epidemiologic and experimental evidence exists, is semen. Semen components include sperm, seminal fluid, lymphocytes, and sometimes infectious agents, including HIV, mycoplasmas, and herpes and hepatitis viruses, all of which independently cause immune suppression. Extensive evidence demonstrates sperm (and various viruses) contains many proteins mimicking the CD4 protein of T-helper cells, while HIV, mycoplasmas, and seminal fluid mimic class II MHC proteins of other lymphocytes. We identify a large number of protein sequences that display such mimicry using computer homology searching, and demonstrate experimentally that sperm antibodies specifically precipitate antibodies against class II MHC mimics such as mycoplasmas, which in turn precipitate antibodies to lymphocyte antigens. These data prove that immunologic exposure to sperm and lymphocytes (as may occur in receptive anal intercourse, needle sharing, or blood transfusions) is theoretically capable of initiating lymphocytotoxic autoimmunity. Such autoimmunity may play a significant role in the pathogenesis of AIDS, and will need to be addressed clinically in high risk individuals regardless of HIV status and regardless of the success of anti-HIV prophylaxis and treatment.

Introduction

AIDS and ICL are due to more than just HIV

An increasing body of evidence suggests that human immunodeficiency virus (HIV) may not be a sufficient, or in some cases (many now classified as idiopathic CD4-T-cell lymphopenia or ICL) even a necessary, cause of acquired immune deficiency syndrome (AIDS). Various co-factors, including other retroviruses, mycoplasmas, herpes viruses, and drugs have been postulated to act as either necessary co-factors or as adjuvants for HIV infections (Sonnabend & Witkin, 1984; Sonnabend, 1989; Holmberg *et al.*, 1988; Lusso *et al.*, 1988; Buimovici-Klein *et al.*, 1988; Montagnier *et al.*, 1990; Root-Bernstein, 1990a, 1990c, 1992a, 1992b; Squinto *et al.*, 1990; Haverkos, 1990; Lusso, Lori & Gallo, 1990; Lo *et al.*, 1991; Duesberg, 1990, 1992; Littlefield, 1992). Moreover, increasing interest is being expressed by AIDS researchers in the possibility that autoimmunity may play a significant role in T-cell depletion (Sonnabend & Witkin, 1984; Andrieu, Even & Venet, 1986; Ziegler & Stites, 1986; Golding *et al.*, 1988; Hoffmann & Grant, 1989; Hoffmann, 1990; Littlefield, 1992; Sonnabend, 1989; Morrow *et al.*, 1991; Root-Bernstein, 1993; Root-Bernstein & Hobbs, 1993; Weiss, 1993; Fauci, 1993). Particularly noteworthy are reports of lymphocytotoxic autoantibodies directed at CD4+ T cells in up to 95% of AIDS and 60% of AIDS related complex (ARC) patients but not in healthy HIV-infected people or HIV-infected chimpanzees and macaques. (Dorsett *et al.*, 1985; Kiprov *et al.*, 1985; Kloster, Tomar & Spira, 1984; Strickler

et al., 1987a, 1987b; Kopelman & Zolla-Pazner, 1988; Zarling *et al.*, 1990; Szabo *et al.*, 1992; Stroncek *et al.*, 1992; Clerici *et al.*, 1993).

Whether lymphocytotoxic antibodies are a cause of AIDS, a pathogenic correlate to AIDS development, or simply one of the many disease manifestations that accompanies immunological breakdown as a result of AIDS is not, at this time, known. Regardless of the role such autoimmune processes play in AIDS, however, it is clear that they, like all autoimmune phenomena, progress independently of their initiating factors and therefore represent a problem of significant clinical concern. The problem of autoimmune phenomena in AIDS is exacerbated by two factors: the complete ignorance of the biomedical research community concerning the cause or causes of any human autoimmune diseases or their effective treatments and the probability that such phenomena will continue to affect AIDS patients regardless of the success or failure of anti-retroviral therapies and prophylaxis. We therefore believe that it is essential that the causes of autoimmune phenomena in AIDS be elucidated and animal models of them developed so that the critical work of finding preventative and curative procedures can have some hope of success.

One among many antigenic stimuli that might act as a possible cause of autoimmunity and co-factor in AIDS pathogenesis are alloantigens found in blood and semen (Hsia *et al.*, 1984; Shearer & Rabson, 1984; Hoff & Peterson, 1989; Hoff *et al.*, 1991; Kion & Hoffmann, 1991; Stott *et al.*, 1991). All groups identified as being at high risk for AIDS are characterized by having repeated immunologic exposure to foreign lymphocytes or their proteins (through exposure to blood or blood products) or to semen (which contains both sperm and lymphocytes). Both Stott *et al.* (1991) and Kion and Hoffmann (1991) have demonstrated the presence of anti-lymphocyte antibodies that cross-react with HIV antigens in HIV-free animals exposed to lymphocytes or with autoimmune diseases such as lupus. Indeed, lymphocytes are common components of both blood and semen (Shearer & Rabson, 1984), and strong evidence has existed from the outset that semen components (sperm and/or lymphocytes) may play a pathogenic role in AIDS. Receptive anal intercourse is the most significant of the risks identified for homosexual and bisexual men (Darrow *et al.*, 1987) and for heterosexual women (The European Study Group, 1989; Padian *et al.*, 1990). The same mechanisms proposed for promoting HIV and other sexually transmitted infections – abrogation of the anal or intestinal tissue due to unprotected anal forms of sex or untreated sexually transmitted or enteric diseases – would also permit sperm cells and foreign lymphocytes to enter the blood stream or lymph system.

Although women who practice anal intercourse and subsequently develop AIDS have not specifically been tested for LCTAs and anti-serum antibodies, nearly all i.v. drug addicts regardless of sex, are positive for anti-MHC antibodies (de la Barrera *et al.*, 1987), and 30% of unselected female AIDS patients in one New York study (that included many i.v. drug abusers) had significantly high antibody to human sperm extract, protamine, and fertilization antigen as compared with healthy controls (Naz *et al.*, 1990). The prevalence of such antibodies in male homosexual AIDS patients is much higher: 40% have significant antibody against human sperm extract, 70% against protamine, and 70% against fertilization antigen (Naz *et al.*, 1990). Such antibodies were not present in any HIV-negative individual tested. The presence of circulating immune complexes (CICs) which included anti-semen antibody components was even higher in each group. CICs were found in 90% of male AIDS serum samples and 80% of female AIDS serum samples (Naz *et al.*, 1990). These figures have been verified by other investigators looking at autoimmune problems associated with AIDS (see autoimmunity references above and anti-sperm antibody references below). The importance of CICs is that they represent sure diagnostic signs of autoimmune phenomena.

The presence of anti-lymphocyte and anti-semen antibodies is of great potential importance for understanding AIDS. Autoimmune processes in AIDS are directed at virtually every organ, system, and tissue in the body (Morrow *et al.*, 1991). Obviously, anti-lymphocyte antibodies may play a significant role in immune suppression, not only against T-helper cells, but other cells in the immune system as well. Moreover, sperm contains a large number of antigens that are cross-reactive with other human tissues, including lymphocytes, kidney and brain (Lewis, 1934; Naz, 1988), and may therefore induce a range of autoimmune disease symptoms. Among these autoimmune effects are T-cell suppression, since anti-sperm antibodies have been demonstrated to cross-react with host T-cells in AIDS risk groups, and to be a direct cause of immunosuppression through T-cell killing (*in vitro*) (Adams *et al.*, 1988; Bagasra *et al.*, 1988; James & Hargreave, 1984; Mathur *et al.*, 1981; Mavligit *et al.*, 1984; Rubenstein *et al.*, 1989; Witkin & Sonnabend, 1983). Indeed, lymphocytotoxic antibodies have been

found not only in HIV-positive, but in some high-risk HIV-negative homosexuals; however, such antibodies are not found in healthy heterosexual controls (Kiprov *et al.*, 1985; Adams *et al.*, 1988).

Anti-sperm antibodies may also be the cause of the anti-testicular and nervous system autoimmunity that is present in virtually all gay men and many women who develop AIDS. Aspermatogenesis, sperm maturation arrest, and testicular fibrosis are the rule rather than the exception in such patients (Reichert *et al.*, 1983; Welch *et al.*, 1984; Niedt & Schinella, 1985; Krieger *et al.*, 1991). Autoimmunity directed at brain proteins is also extremely common, if not universal among AIDS patients (Morrow *et al.*, 1991), and interestingly the connection between sperm LCTAs and brain antigens has been verified by studies of people who develop LCTAs following spinal cord damage; these LCTAs cross-react with sperm, and can cause aspermatogenesis (Hirsch *et al.*, 1992; Tsatsoulis & Shalet, 1991). Thus, anti-sperm immunity can result in a wide range of immunologically-based pathologies associated with AIDS.

Experiments in animals have confirmed that immunological exposure to semen, including anal modes of presentation, can result in T-cell immunosuppression as measured by T-cell counts and activity assays (Hurtenbach & Shearer, 1982; Richards, Bedford & Witkin, 1983). These animals were not exposed to opportunistic infectious agents, so it is not known whether their immune suppression was significant enough to result in AIDS, or merely represents a predisposing factor or cofactor for AIDS development. Similar experiments have shown that seminal plasma also contains proteins that directly inhibit T-cell blast transformation, natural killer (NK) cell and macrophage activity, and response to foreign antigens. (Lord, Sensabaugh & Stites, 1977; Anderson & Tarter, 1982; James *et al.*, 1983; Williamson, 1984; Schopf *et al.*, 1984; Erikson, 1984; Marcus *et al.*, 1987; Quayle *et al.*, 1987; Naz *et al.*, 1990).

Most importantly for the possible role semen may play as an inducer of lymphocytotoxic autoimmunity in AIDS and ICL, sperm contains proteins that immunologically mimic lymphocyte proteins (Naz, 1988). Gobert *et al.* (1990) have demonstrated that anti-CD4 monoclonal antibodies react with several different proteins in sperm membranes, sperm extract, and seminal fluid, and significant regions of homology between CD4 protein and sperm surface proteins have been reported (Root-Bernstein & Hobbs, 1993). Ashida and Schofield (1987) have shown that sperm will therefore bind to class II MHC receptors on lymphocytes. Conversely, Bagasra *et al.* (1988) have demonstrated that HIV (which mimics class II MHC activity by binding to CD4 receptors on T cells) will bind to the CD4-like epitopes on sperm. Interestingly, Mycoplasmas, which share large homologous regions of class II MHC-like sequences with HIV gp120 protein (Root-Bernstein & Hobbs, 1991, 1993), also bind to sperm (Black, 1983). Thus, it is likely that many immunogenic agents associated with AIDS 'piggyback' on each other, so that sperm, HIV, Mycoplasmas, and other disease agents associated with AIDS may all be transmitted as complexes that act synergistically when immunologically processed (Root-Bernstein & Hobbs, 1993). Additionally, Autiero, Abrescia and Guardiola (1991) have discovered class II MHC-like seminal proteins that bind directly to CD4-like sperm proteins, and which may therefore themselves mimic the antigenicity of both class II MHC lymphocytes and HIV gp120 protein. Since anti-CD4 antibodies and anti-HLA antibodies are both extremely common forms of LCTAs in AIDS patients (Shearer & Rabson, 1984; Shearer, 1984; Sonnabend, 1989; Morrow *et al.*, 1991), these data concerning the antigenic similarities and cross-reactivities of semen components with lymphocytes are striking clues as to the possible causes of at least some of the lymphocytotoxic autoimmunity found in AIDS and ICL.

Theory

The role of homologies in the induction of autoimmunity

Given these facts, we surmised that proteins found in sperm might induce lymphocytotoxic autoantibodies, and therefore that significant homologies might exist between semen proteins and proteins of lymphocytes including but not limited to CD4. Simple homology between self and nonself proteins, combined with exposure to the nonself protein, is not, however, sufficient to induce autoimmunity (Westall & Root-Bernstein, 1983, 1986; Root-Bernstein, 1991).

The basic phenomenon of autoimmunity is that the immune system, which normally protects the body from disease, turns upon the body itself in a sort of immunologic civil war. The response can be mediated by B cells or T cells, or both. In most cases, the resulting immunological civil war develops over many years (as, notably, does AIDS), and is

progressively debilitating. Well-known, slowly progressing, often fatal examples of autoimmune diseases are multiple sclerosis, systemic lupus erythematosis, and amyotrophic lateral sclerosis, For reasons that are not understood, the part of the immune system that distinguishes between self and non-self fails, and the immune system targets its own tissues or organs for destruction. One reason that no one understands autoimmunity is that no one has yet put forward a cogent theory of how the immune system distinguishes between self and non-self. One thing all theories of autoimmunity agree upon is that self-nonself distinction is partially lost during autoimmunity, and that mimicry between the inducing agent or agents and self tissues is at the root of the failure of the immune system to distinguish between self and non-self (reviewed in Root-Bernstein, 1991).

There are three basic types of theories about how autoimmune processes are initiated (reviewed in Root-Bernstein, 1991). The first type can generally be called the 'antigenic mimicry' theory (Fujinami & Oldstone, 1985; Oldstone, 1987; Bjork, 1991; Susal *et al.*, 1993). According to this type of theory, infectious agents may contain proteins that sequentially or structurally mimic proteins within the host body. Under normal circumstances, these antigenic mimics will not provoke an immune response from the host because the host immune system 'recognizes' that to do so would result in an autoimmune reaction as well. Under some conditions (which are never specified in these theories), the self/non-self distinction is abrogated. It is generally asserted that this loss of self/non-self distinction occurs most often if the antigen is different enough from the host protein to elicit an immune response, but similar enough to allow functional cross-reactivity. In AIDS, a relevant example is the well-established antigenic and functional mimicry of HIV gp160 with MHC class II proteins (Bjork, 1991; Dalgleish *et al.*, 1992; Susal *et al.*, 1993). Alloantigens are another source of antigenic mimics.

A second type of autoimmune theory proposes that the cause is not the primary response to an antigen, but the anti-idiotype response (Plotz, 1983; Williams *et al.*, 1989; Hoffmann & Grant, 1989). Typically, the immune system mounts a primary antibody response against an antigen. When the antigen is eliminated from the host, a secondary immune response is elicited in which the primary, or idiotypic antibody, induces a chemically complementary antibody, known as the anti-idiotype antibody. According to Jerne's network theory of immunologic control, as series of such idiotype/anti-idiotype networks is what regulates immune function. In order to induce autoimmunity, anti-idiotype theories suggest that the following sequence of events occurs. A virus (or other infectious agent) uses a host cellular receptor to enter a cell. The viral coat proteins are chemically complementary to this cellular receptor. (An example relevant to AIDS would be the use of gp160 binding to CD4 receptors to infect macrophages, dendritic cells, and T-helper cells.) According to the anti-idiotypic autoimmunity theory, the primary antibody response will be to the foreign antigen gp160. This idiotypic antibody will have the same general specificity as CD4. The idiotypic antibody will, in turn, induce an anti-idiotypic antibody which will now mimic the binding properties of the original antigen, gp160. Thus, this anti-idiotypic antibody will recognize as its target, the same cellular receptor as its target, as does the virus (Hoffmann & Grant, 1989). In this way, viruses might be able to induce autoimmune processes against their target tissues. Similarly, anti-CD4 autoimmunity might be provoked by alloantigenic exposure to MHC class II proteins following exposure to blood or semen (Kion & Hoffmann, 1991; Stott *et al.*, 1991).

A number of important difficulties exist with both the antigenic mimicry and anti-idiotype theories of autoimmunity. First, neither specifies how or under what circumstances the self/non-self distinction will be abrogated. Indeed, the anti-idiotype theory asserts that the very network of idiotype/anti-idiotype interactions that is supposed to regulate immune function is the cause of autoimmunity! Second, molecular mimicry, although undoubtedly necessary to induce a cross-reactive autoimmune response, cannot be sufficient. More than 3% of monoclonal virus antibodies have been found to cross-react with human tissues (Srinivasappa *et al.*, 1986), and we are infected with hundreds of viruses during our lifetimes. If simple antigenic mimicry, whether idiotypic or anti-idiotypic, were sufficient to induce autoimmunity, we should all end up with multiple autoimmune diseases following every viral infection. The actual incidence of any individual autoimmune disease is on the order of one in tens or hundreds of thousands and the cumulative incidence only a few percent of the population, even if we include the arthritic diseases. Thus, something mediates between the commonness of antigenic mimicry and the rarity of autoimmunity. Finally, simple exposure to purified alloantigens and molecular mimics does not lead to autoimmunity in experimental and natural conditions (reviewed in Root-Bernstein, 1991). If

it were otherwise, vasectomies or uncomplicated vaginal intercourse would each carry a significant risk of the development of anti-semen antibodies that could induce autoimmune conditions. As far as is known, they do not. For example, LCTAs do not result in human beings from true auto-exposure to sperm, such as that following vasectomy (Jennings, Wettlaufer & Paulsen, 1977).

A third theory of autoimmunity has therefore been proposed that satisfies all known data from animal experimentation, explains how the self/non-self distinction is abrogated, and accounts for the extreme rarity of autoimmune processes in the general population while also explaining why it is so high among people with AIDS. This third theory is called the multiple-antigen-mediated autoimmunity (MAMA) theory (Westall & Root-Bernstein, 1984, 1986; Root-Bernstein, 1990b, 1991, 1993). It suggests that autoimmunity is due to more than simple cross-reactivity following antigenic processing of self-mimicing antigens. Such self-mimicry is a necessary, but insufficient condition for inducing autoimmunity according to MAMA. MAMA requires a pair of antigens which are chemically complementary, at least one of which is a self-mimic. It therefore predicts that autoimmunity is most likely to occur in people infected with or exposed to multiple, concurrent allogeneic or infectious antigens.

MAMA was proposed in 1984 by Westall and Root-Bernstein (1984, 1986) to explain animal models of multiple sclerosis (experimental allergic encephalomyelitis), experimental autoimmune orchitis, and experimental thyroiditis. In each of these experimental autoimmune diseases, two chemically complementary antigens (Root-Bernstein & Westall, 1990) are necessary to induce the disease. Neither antigen can induce autoimmunity by itself. Each antigen induces an antibody (or T-cell) response specific to it. Since the antigens are chemically complementary, some of the respective antibodies or T-cells are also complementary – or in immunological terms, they are idiotype/anti-idiotype pairs (Fig. 1). Each antibody (or T-cell) now recognizes the other as mimicing an antigen. Self/non-self distinction is therefore abrogated by the simple act of producing an immune response to each antigen. If one or both of the antigens also mimics a self-protein, then part of the immunological civil war that results will be directed at a tissue target as well (Westall & Root-Bernstein, 1983, 1986).

There is an additional constraint imposed on the induction of autoimmunity by the MAMA mechanism,

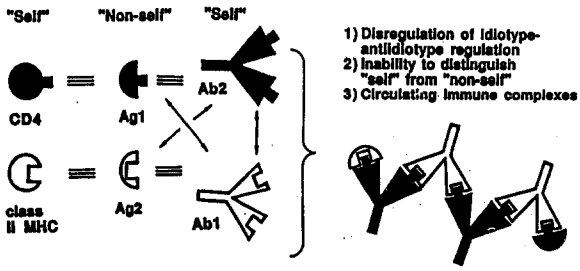

Fig. 1. The immunologically disruptive effects of processing two, chemically complementary antigens (Ag1 and Ag2) that mimic the complementary lymphocyte proteins CD4 and class II MHC (HLA class II). The resulting antibodies (Ab1 and Ab2) are chemically complementary to their antigens, but are also chemically complementary to each other, therefore representing an idiotype/anti-idiotype pair (though each is truly an idiotypic antibody). As a result of molecular mimicry, self and non-self antigens become confused (CD4, Ag1, and Ab2 are all mimics, as are class II MHC, Ag2, and Ab1). Inability to distinguish self from non-self results, allowing the immune system to attack the body; disregulation of the idiotype/anti-idiotype system results, so that the autoimmune process is unregulated, and circulating immune complexes made up of Ab1, Ab2, Ag1 and Ag2 are formed.

which is also bolstered by extensive experimental evidence. As noted above, the antigenic stimuli must be present concurrently. It has been shown both theoretically and experimentally that if one of the pair of complementary antigens is present significantly prior to the other, so that a full immune response is in place prior to exposure to the second antigen, then no autoimmunity will be induced. On the contrary, it has been extensively demonstrated that any individual antigen from a pair can vaccinate against the autoimmunity induced by the corresponding antigen pair (reviewed in Westall & Root-Bernstein, 1984, 1986; Root-Bernstein, 1991).

We emphasize that the outcome of the MAMA theory is virtually identical to an idiotype/anti-idiotype theory of autoimmunity proposed previously by Hoffmann to explain AIDS-associated autoimmunity (Hoffmann & Grant, 1989; Hoffmann, 1991). The major difference between the theories is that MAMA requires two chemically complementary antigenic inducers, whereas Hoffmann believes that a single antigen can induce autoimmunity through the simple anti-idiotype mechanism described above. (See Root-Bernstein, 1991 and 1993 for a more detailed explanation for why we do not believe that Hoffmann's theory is tenable.)

Epidemiologic evidence supports the MAMA theory. According to the MAMA theory, the reason autoimmune conditions are thousands of times more

common in AIDS patients than in the general population (reviewed in Marrow *et al.* 1991) is that people with AIDS have an extremely high incidence of multiple, concurrent infections combined with alloantigenic stimuli (Root-Bernstein, 1991, 1993; Root-Bernstein & Hobbs, 1993). We believe that specific *combinations* of antigens are necessary to induce the various autoimmune diseases associated with AIDS and have previously demonstrated that a combination of active herpes virus infection with a mycobacterial infection is extremely high correlated ($p > 0.01$) with the incidence of autoimmune demyelinating disease in both AIDS and non-AIDS patients (Root-Bernstein, 1990b).

The application of the MAMA theory of T-cell directed autoimmunity to AIDS is similarly compelling (Root-Bernstein, 1991, 1993; Root-Bernstein & Hobbs, 1993). The major targets of autoimmunity in AIDS are T-helper cells and class II MHC. These cells are known to have chemically complementary proteins on their cell surfaces: the CD4 protein of T-helper cells and macrophages, and the class II MHC proteins on HLA-bearing cells. The interaction of these two molecules in the presence of antigen is necessary to initiate immune responses. We have summarized above significant data showing that various components of semen, including sperm itself, seminal fluid, and lymphocytes contain CD4-like antigenic sequences. All that is necessary to satisfy the conditions for inducing autoimmunity set out by our hypothesis is that sperm be presented to the immune system at the same time as class II MHC-like antigens, and evidence summarized above demonstrates that these are also often present in semen in the form of HIV antigens (gp120) and mycoplasma infections, and possibly class II MHC-bearing lymphocytes. Thus, it is very likely that the semen of people with AIDS will, unlike the semen of the vast majority of the male population, contain both CD4 and class II MHC-like antigens that may be sufficient to induce autoimmunity directed at lymphocytes bearing both sorts of proteins in anyone immunologically exposed to their semen.

Before going further, we must stress the point that autoimmunity will result only from *immunologic* exposure to the semen-infection combination. Antigenic exposure to semen during vaginal intercourse is extremely rare because there are no lymph nodes in the immediate vicinity, few lymphocytes within the vagina or cervix, and no access to the blood stream during the normal course of events. Thus, antibody responses to semen components in women without AIDS are extremely rare, and of these responses almost none are of the lymphocytotoxic type, but are rather directed at non-mimicry regions of semen. Furthermore, even if semen were to acquire access to the blood or lymph system, we reiterate that it would provoke an autoimmune response only if it were immunologically processed with an appropriate antigenic cofactor (allogeneic or infectious).

One prediction unique to the MAMA theory is easily testable and positive results would help to establish its plausibility (although it certainly will not prove it). The theory uniquely predicts that since one antigen mimics CD4 (or a similar T-cell antigen) and the other antigen mimics class II MHC (or a similar lymphocyte antigen), then the antibodies against this pair of antigens should be chemically complementary. In other words, the two separate antigens should induce idiotype/anti-idiotype antibody pairs. These idiotype/anti-idiotype antibody pairs (actually two interacting idiotypes) will precipitate each other. Furthermore, the theory requires that one or both of these antibodies be capable of cross-reacting with a self antigen. This cross-reactivity will also be evident as precipitation of antibody raised to this cross-reactive antigen, due to complementarity. The result will be the *in vitro* equivalent of the circulating immune complexes (CICs) that typify the autoimmune processes present in nearly all AIDS patients (see above and Fig. 1). Since the inducing antigens mimic both CD4 and class II MHC proteins, the loss of self/non-self distinction will allow the immune response to the antigens to become autoimmune in nature. In this instance, the resulting immunologic civil war will be directed against CD4 and class II MHC lymphocytes, resulting in T-cell loss and non-specific immunologic activation (Fig. 2). No other theory of autoimmunity predicts these sorts of idiotype/anti-idiotype interactions (with the possible exception of the Hoffmann theory), and therefore evidence of the type of mutual antibody precipitation predicted here will uniquely support the MAMA theory.

Methods

Protein similarity searches

In order to test the plausibility of semen as a plausible inducer of autoimmunity, we performed two types of studies. The first involved computer-based searches for protein similarities between semen-associated proteins

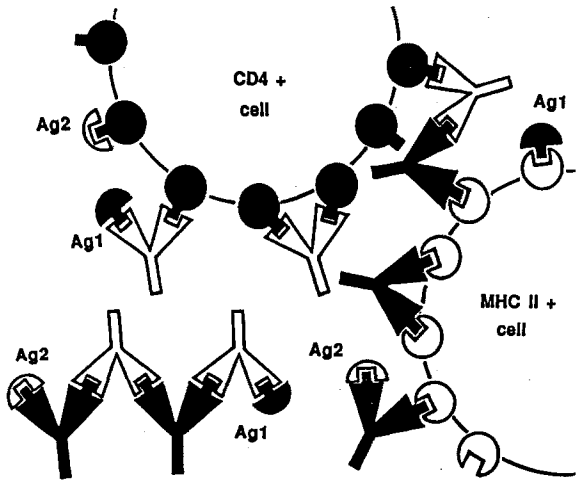

Fig. 2. As a result of the process begun in Fig. 1, Ab1 can attack Ag1 and its self mimic, CD4; Ab2 can attack Ag2 and its self mimic, class II MHC; and immune complexes can form between CD4 and class II MHC cells, causing syncitia. In addition, circulating immune complexes will form complexes between CD4 proteins and Ag2, as well as between class II MHC and Ag1. These antigen-lymphocyte-receptor complexes and antibody-lymphocyte-receptor complexes would result in non-specific activation of immunity, as is universally observed in AIDS.

and lymphocyte proteins. Positive evidence of protein sequence similarities between sperm and lymphocyte antigens and cellular targets of autoimmunity in AIDS would support the plausibility of molecular mimicry being the basis of autoimmune reactions in AIDS. It would not, however, distinguish between autoimmune theories. The second test is therefore specifically to differentiate the MAMA theory from other autoimmune theories. It consists of a preliminary screen of antibodies against sperm, lymphocytes and infectious agents found in the semen of AIDS patients that might interact in the complementary manner proposed above to produce an *in vitro* model of CICs.

We performed homology searches utilizing two methods available in the Genetics Computer Group Sequence Analysis Software package (Devereux, Haeberli & Smithies, 1984). A Wilbur and Lipman (1983) style WORDSEARCH of the Swiss-Prot Protein Sequence Database (18,364 proteins) was accomplished using a word size of 4 (4-mer) with no mismatches and a word size of 5 (5-mer) allowing one mismatch followed by alignment of the 400 best diagonals of each of these analyses. Additionally, a Pearson and Lipman (1988) FASTA analysis of the Swiss-Prot Protein Sequence Database using a word size of 2 (2-mer) followed by alignment of the 400 best diagonals was also performed. The resulting printouts were then analyzed in light of their potential relevance for the hypothesis under consideration. Homologies were screened for significance using the following criteria: an identical amino acid was given a score of one; acceptable substitutions were given a score of one half; a homology was considered significant only if a sequence achieved a score of at least five within a sequence of ten amino acids (i.e., at least a 50% identity in a 10-mer). 10-mers were chosen as reasonable lengths for evaluation since peptides of this length are more or less what are recognized by T-cell receptors (Rudensky *et al.*, 1991). In fact, the reported sequences, in general, are greater than ten amino acids in length and achieve much higher degrees of similarity than we required.

Controls were run to determine the degree to which random matches between proteins might occur. Whereas six similar sequences between CD4 and sperm proteins appeared in the 400 best fits when the CD4 sequence was run against the entire Swiss-Prot data base, no similarities were found among the top 400 best fits when CD8, human serum albumin, nerve growth factor, or the three T-cell receptor subunits (alpha, beta, and gamma) were run against the data base. We therefore interpret these negative data to mean that the similarities we are reporting between CD4 and sperm proteins are significantly unusual.

We make no claim to completeness. Significant homologies may exist between lymphocyte proteins and semen proteins other than the ones we report here, particularly as the protein sequence data base is continuously growing. Due to the strict bias employed in evaluating the data, the large amount of corroborative data achieved by these methods, and due to previous work that has found a very large number of additional similarities specifically between lymphocyte proteins and *cell surface* proteins of sperm that are not reported here (Root-Bernstein & Hobbs, 1993), no additional searches were pursued. Only similarities not published previously are listed in the present paper.

Immunodiffusion studies

The specific predictions of the MAMA theory regarding coprecipitation of complementary antibodies to pairs of complementary antigens (Fig. 1) was tested using a modification of standard Ouchterlony immunodiffusion (Ouchterlony, 1968), in which antibody is diffused through an agarose gel against other antibodies instead of against antigens. We term this modified

technique double antibody diffusion, or DAD (Root-Bernstein, 1994).

We obtained immune serum (sheep) raised against human sperm from two suppliers (Chemicon; Arnel) and seventy-five polyclonal (PAB) and monoclonal (MAB) antibodies against human lymphocyte proteins, bacteria, and viruses associated with AIDS. These antibodies included 14 mycoplasmas, 4 mycobacteria, 2 Streptococci, 1 Staphylococcus, 1 *E. coli*, 2 *Candida albicans*, 8 HIV, 8 cytomegalovirus (CMV), 3 hepatitis B virus (HBV), 3 herpes simplex virus (HSV), 4 Epstein-Barr virus (EBV), 1 measles, 1 adenovirus, 6 HLA class I and II, 3 T-helper lymphocytes, 1 T-suppressor, 5 monocyte/macrophage, 1 natural killer cell (NK cell), 4 blood clotting factors, and 1 platelet. Specifically, the following antibodies were tested using the DAD technique: *Mycoplasma fermentans*, strain incognitus MAB and rabbit PAB, courtesy Dr. Shyh-Ching Lo, Armed Forces Institute of Pathology, Washington, DC; M. fermentans strain K7 (rabbit) and strain PG18 (mule), *M. pneumoniae* strain FH (mule), *M. genetalium* strain G37 (rabbit), *M. pirum* strain 70–159 (rabbit), *M. hominis* strain PG21 (mule) and strain MY13330 (rabbit), *M. orale* strain CH19299 (mule), *M. salivarum* strain PG20 (mule), all courtesy of Dr. Joseph G. Tully, NAIAD, Bethesda, MD; MAB to human T-helper cell (AXL839M), MAB to CD13 monocyte antigen (YN-3D8), MAB to Factor VIII (AXL738M), PAB to Factor IX (AXL423), polyclonal antibody to Factor V (AXL422), MAB to von Willebrand factor (Factor VIII-related antigen) (AXL739M), MAB to platelet glycoprotein 1b (AXL842M), MAB to T4 helper-inducer cells (AXL839M), MAB to T suppressor-cytotoxic cells (AXL830M), MAB to macrophage antigen (AXL841M), MAB to CD13 (monocyte marker) (YNW-3D8-B), MAB to HLA-DR (AXL827M), PAB to CMV (BX-PU083-UP), PAB to HBV surface antigen (AXL-683), PAB to HBV core antigen (AXL709), PAB to HSV 1 and 2 (086P), PAB to HSV 1 (Macintyre, VR3) (AXL237), PAB to HSV 2 (MS) (AXL239), PAB to *M. bovis* (AXL247), *M. duvallii* (AXL248), and *M. paratuberculosis* (AXL435), and PAB to *E. coli* (AXL480), all from Accurate Chemical and Scientific Corporation, Westbury, N.Y.; MAB to human CD4 gp55kD (P42115M), MAB to human monocyte-macrophage CD11c (P90105E), MAB to human follicular dendritic cells (P90106C), MAB to human HLA DQw1 (P61410M), MAB to HLA class II (DQ) (P42416M), MAB to HLA-DRw52 (P61510M), MAB to human HLA class II DP + DQ + DR (P90103E), MAB to HIV gp160/120 (C42869M), MAB to HIV 1 (C45310M), goat anti-HIV-1 (B65870G), goat anti-HIV-1, IgG (B65875G), sheep HIV-1 gp120-1 (B1289S), MAB to CMV 65 kD major matrix protein (C65083M), chicken anti-CMV AD169 (B85275C), goat anti-CMV (B65270G), rabbit anti-CMV (B47821R), rabbit anti-Streptococcus type A (B66885R), rabbit anti-Streptococcus type B (B66880R), rabbit anti-Mycoplasma hyorhinus (B80720R), rabbit anti-Candida albicans IgG (B17401R), MAB to Epstein-Barr virus (C65221M), MAB to HBV surface antigen S protein (C42863M), all from Biodesign International, Kennebunkport, Maine; MAB to human natural killer cells (NK cells) (MAB1221), MAB to HLA common marker (MAB1275), MAB to HIV gp120 IgG2a (MAB883), MAB to HIV gp41 (MAB882), MAB to CMV late antigen (MAB8125), MAB to CMV IgG1,k (MAB810), MAB to *M. bovis* (MAB970), MAB to *M. pneumoniae* (MAB828), MAB to *M. avium* (MAB968), MAB to *Candida albicans* (MAB806), MAB to EBV viral capsid antigen (MAB817), MAB to EBV membrane antigen IgG1k (MAB813), MAB to adenovirus (MAB8051), MAB to measles blend (MAB8920), MAB to *Varicella zoster* IgG2b (MAB8612), MAB to *Varicella zoster* IgG1 (MAB8614), MAB to EBV early antigen (MAB818), MAB to human HIV Nef (MAB899), MAB to *Staphylococcus aureus* (MAB930), all from Chemicon, International, Inc., Temecula, California.

Antibodies and sera were used at the concentrations supplied (usually ca. 1 mg of protein per ml). Diffusions were carried out using standard 5% agarose gels made with deionized water with Tris buffer. Precipitations were read without staining the gels. DAD was performed on all possible combinations of the sperm sera with the 75 other antibodies, and all combinations were run at least twice and in the case of positive results, three times. Over 1800 of the some 2500 possible combinations of the other 75 antibodies have also been run at least in duplicate, limited only by the amount of antibody available within our budgetary constraints.

Results

Protein similarity studies

The results of our search are summarized in Tables 1 through 4. Table 1 displays the homologies found

Table 1. Similarities between human CD4 protein and sperm nuclear proteins.

1.	CD4 9–28	HLLLVLQLALLPAATQGKKV
		|III |II |||| |I I
	H2B 98/99–117–118	RLLLPGELAKHAVSEGTKAV
	Human histone H2B and H2B.1	
2.	CD4 20–36	PAATQGKKVVLGKKGDT
		||I||I II IIII |
	HMG-17 43–59	APAKKGEKVPKGKKGKA
	Human HMG-17 non-histone chromosomal protein	
3.	CD4 156–181	RSPRGKNIQGGKTLSVSQLELQDSGT
		I|||II| | | | ||I ||||I
	HMG-14 55–80	RGAKGKQAEVANQETKEDLPAENGET
	Human HMG-14 non-histone chromosomal protein	
4.	CD4 203–223	AFQKASSIVYKKEGEQVEFS
		| II | | II|I I
	H2B1 13–22	GSKKAVTKAQKKDGKKRKRS
	Human histone H2B1 (these sequences are nearly identical in all H2B histones)	
5.	CD4 230–250	EKLTGSGELWWQAERASSSKS
		III || |II||I / II I
	HMG-17 310–330	EKLPQEAMVWWKEEAERSSSS
	Human HMG-17 non-histone chromosomal protein	
6.	CD4 265–277	RVTQDPKLQMGKK
		/ II| I | III
	H2B1 15–28	KKAVTKAQKKD.GKK
	Human histone H2B1 (these sequences are nearly identical in all H2B histones	
7.	CD4 279–305	PLHLTLPQALPQYAGSGNLTLALEAKT
		|||I II I| I I| I I| / I
	H2B1 97–122	AVRLLLPGELAKHAVSEG.TKAVTKYT
	Human histone H2B1 (these sequences are nearly identical in all H2B histones)	
8.	CD4 421–443	RCRHRRRQAERMSQIKRLLSEKK
		/ IIIIII| / || II ||||
	PROTAMINE 79–100	RSCRHRRRHRRGCRTRKR..TCRR
	Human sperm histone p2 precursor (protamine p2)	
9.	CD4 421–437	RCRHRRRQAERMSQ.IKR
		I|I|III| || | I
	PROTAMINE 21–37	RSRRRRRRSCQTRRRAMR
	Human sperm histone p1 protein (protamine p1)	
10.	CD4 421–447	RC.RHRRRQAERMSQ..IKRLLSEKKLLSE
		|| ||I|I| I || I | I|| |
	H2B1 24–51	KDGKKRKRS..RKESYSIYVYKVLKQVHPD
	Human histone H2B1 (these sequences are nearly identical in all H2B histones)	

between CD4 protein, protamine, high mobility group (HMG) chromosomal protein 17, and histone H2B1. Protamine P1 is a histone-type protein found only in sperm (Meistrich, 1989). As noted above, 70% of male homosexual and 40% of female AIDS patients in one study had antibody against protamine (Naz *et al.*, 1990). HMG-17 is a DNA-associated structural protein. Its role has not been established (Bustin *et al.*, 1989) and no information appears to exist regarding its possible role in AIDS. Histone H2B is found in many tissues including thymus (which is the essential organ for T-cell activation), and is unusual in also being found in sperm. Although in general most histones are eliminated from the nucleus during sperm formation,

there are two major exceptions, and those are histones H1t and H2Bt (Tanphaichitr *et al.*, 1978; Wattanaseree & Svasti, 1983). Unfortunately, the specific sequence of H2Bt was not available for homology searching, but all of the H2B family of histones maintain highly conserved sequences so that little, if any error, has been introduced (Gabrielli, 1989, Fig. 1).

The observation that histone H2B has significant homologies with CD4 is very interesting in light of a discovery by Stricker *et al.* (1987a, 1987b) of an autoantibody in the majority of AIDS patients that not only recognizes an unidentified antigen on CD4+ cells, but also strongly cross-reacts with histone H2B. Morrow *et al.* (1989; 1991) report that these anti-histone antibodies correlate with disease activity and loss of CD4+ cells in longitudinal studies of AIDS patients.

It is also noteworthy that herpes simplex virus (O'Hare & Goding, 1988; Latchman *et al.*, 1989; Rhys *et al.*, 1989), cytomegalovirus (Munch *et al.*, 1988), and adenoviruses (O'Neill & Kelly, 1988) all require H2B histone-associated binding sites on DNA for activation and have been found to trans-activate an octomeric histone H2B promoter, leading to the observation that ubiquitinated histone H2B is preferentially expressed and located on transcriptionally active chromatin (Nickel, Allis & Davie, 1989). This functional overlap suggests that antibody to H2B may result not only from exposure to sperm, but also either directly or indirectly from virus infection.

A further homology search using H2B as the search string yielded two other possible cross-reactive targets on blood cells for antibody to H2B: CD7 protein, and beta platelet growth factor receptor precursor protein (Table 2). Notably, CD7 protein is an early T-cell specific membrane antigen that is associated with production of immature cells in leukemia (Haynes *et al.*, 1980; Lo Coco *et al.*, 1989; Thiel *et al.*, 1989). In the context of AIDS, autoantibody against CD7 might result in failure of T-cells to mature, causing significant loss of not only CD4 cells but of other T-cell subsets as well. Autoantibody against platelet growth factor receptor (Table 2) might contribute to the thrombocytopenia that is very common among AIDS patients (Morrow *et al.*, 1991).

In addition to chromosomal proteins, sperm contains a very high content of mitochondria. These mitochondrial proteins would present another set of possible antigens during antigenic presentation of semen. It is not surprising to find, therefore, that both mitochondrial membrane proteins and several enzymes involved in the various energy pathways contained in the mitochondria have significant homologies with CD4 protein (Table 3). Particularly noteworthy are the similarities between CD4 and creatine kinase, which is an enzyme required not only for mitochondrial activity, but for muscle contraction as well. Thus, anti-CD4 antibody, whatever its source, may cross-react with both mitochondrial and muscle creatine kinase to produce weakness and muscle wasting – two prominent symptoms of ARC and AIDS. In the event, these sequence similarities strongly argue that mitochondrial proteins could potentially activate an autoimmune response cross-reactive with CD4+ cells, and may account, at least in part, for the frequency of antibody observed to 'sperm extract' (Naz *et al.*, 1990).

It is important to note that non-protein components of sperm have also been associated directly with the induction of LCTAs and could not be found using our search techniques. Human DNA, such as might be encountered following repeated exposure to allogeneic sperm, can induce LCTAs (Shoenfeld *et al.*, 1985) and anti-DNA antibodies are very common in AIDS and lupus patients (Morrow *et al.*, 1991). Oddly, the resulting anti-DNA antibodies cross-react specifically with lymphocyte *membranes* rather than with the nucleus (Shoenfeld *et al.*, 1988). Equally oddly, DNA has been found on the cell surfaces of only two kinds of human cells: lymphocytes and cancer cells (Lerner, Meinke & Goldstein, 1971; Aggarwal *et al.*, 1975). In consequence, lymphocyte-associated membrane DNA may provide a target for anti-DNA antibodies, so that they may also act as lymphocytotoxic antibodies in diseases such as AIDS and lupus.

When interpreting the data presented in Tables 1–3, it is also important to bear in mind that sperm is only one component of semen. Semen also contains a large number of lymphocytes. Kion and Hoffman (1991) and Stott *et al.* (1991) have both demonstrated in animal models of AIDS that lymphocytes can induce anti-MHC antibodies that cross-react with HIV. More importantly, whole blood transfusions and granulocyte transfusions have led to direct induction of LCTAs directed at both B cells and T cells in a large proportion of human transfusion patients (Arnold, Goldmann & Pfleiger, 1980; Fehrman, Ringden & Moeller, 1983). This result argues strongly for the likelihood that semen-associated lymphocytes can also produce LCTAs if immunologically processed. There should be no need to have to argue that such allogeneic lymphocytes have significant homologies with host lymphocytes.

Table 2. Similarities between sperm histone H2B1 and Lymphocyte proteins other than CD4.

1.	H2B1 96–117	AVR.LLLPGELA.KHAVSEGTKA
		| I IIII II III II I
	CD7 4–27	PPRLLLLPLLLALARGLPGALAA
	Human CD7 antigen precursor (gp40) (T-cell leukemia antigen)	
2.	CD4 3–24	RGVPFRHLLLVLQLALLPAATQ
		III| ||I | I| | II|III
	CD7 315–336	RGVATQRLCVCLRPPPLPTATQ
	Human CD7 antigen precursor (gp40) (T-cell leukemia antigen)	
3.	H2B1 93–118	IQTA.VRLLLPGELAKHAVSEGTKAVT
		|||| IIII II|| | I | |I
	PGDR 311–335	VESGYVRLL..GEVGTLQFAELHRSRT
	Beta platelet-derived growth factor receptor precursor	

Table 3. Similarities between CD4 protein and mitochondrial proteins.

1.	CD4 100–123	LKIEDSDTYICEVEDQKEEVQLLV
		IIII| ||III| I| I ||I |
	PDH 1878–1898	LKIEKLKXYICTEETIK.VFXLGL
	Hyman pyruvate dehydrogenase beta subunit gene, complete cds	
2.	CD4 204–226	AFQKASSIVYKKEGEQVEFSFPL
		II|| ||| II||||| |IIII
	ATPase 867–889	AFPRWTRVPYKRKEAXLRLSFPL
	Human ATP synthase beta subunit gene, exos 1–7	
3.	CD4 265–280	RVTQDPKLQMGKKLPL
		|II I | ||I | I
	MMP1 73–87	KVTKDG.VTVAKSIDL
	Mitochondrial matrix protein p1 precursor	
4.	CD4 269–286	DPKLQMGKKLPLHLTLPQ
		| II||I||| || |III
	KCRS 398–415	EKKLERGQDIKVPPPLPQ
	Human creatine kinase, sarcomeric mitochondrial precursor	
5.	CD4 307–354	KLHQEVNLVVMRATQLQKNLTCEVWGPTSPK..LMLSLKLENKEAKVSR
		|I II | I|||I III I| | | ||I I |I||| |I
	KCR 295–356	RLIQERGWEFMWNERLGYILTCPSNLGTGLRACVHIKLPLLSKDSRFPK
	Human creatine kinase, mitochondrial precursor	
6.	CD4 438-448	LLSEKKTC..QCP
		IIIIII | I
	MMP1 245–255	LLSEKKISSIQ
	Mitochondrial matrix protein p1 precursor	

To summarize, a wide range of semen components, including sperm surface and nuclear proteins, mitochondrial proteins, seminal fluid, lymphocytes and nuclear DNA, may all contribute to autoimmunity directed at T-lymphocytes. The protein similarity searches confirm that similarities between relevant sperm antigens and CD4 or CD7 proteins (and possible cross-reactivity with other human proteins) may help to account for subsequent immune suppression.

Immunodiffusion (DAD) studies

The results of the DAD experiments also confirm the plausibility of the MAMA hypothesis as applied to the induction of lymphocytotoxic autoimmunity associated with AIDS and ICL. Several of the interactions predicted by the similarity search were observed.

Although over 1800 combinations of antibodies were tested in duplicate or higher repetitions, only results relevant to the question of semen-associated autoimmunity are being reported in this paper, and comprise a subset of 150 tests with sperm antibodies and 294 between Mycoplasma and lymphocyte antibodies, which turned out to be particularly relevant to the current study. Complete results of the remaining combinations are being reported elsewhere (Root-Bernstein, 1994). Suffice it to say that only 75 repeatable positive interactions have occurred among the 1800 non-sperm-antibody combinations tested. While this means a 4% positive rate, this rate is misleadingly high, since the antibodies chosen for testing are not random, but rather were used because of their probability of interacting. Furthermore, a positive interaction between, say, one CMV and one Mycoplasma antibody pair was almost always accompanied by further CMV-Mycoplasma antibody pairs (8 CMV and 15 Mycoplasma antibodies were tested). Thus, each positive result was actually accompanied by several additional confirmational results, artifactually boosting the apparent positive rate. We therefore argue that our data demonstrate clearly that the positive results are due to specific interactions.

Further evidence for specificity is provided by evidence against general precipitation of antibodies raised in particular host species, as might be expected if the binding sites were on the structural rather than the hypervariable (or antigen-binding) region of the antibodies. No patterns of positive results were observed by host species in which the antibodies were raised. Although several of the observed interactions (e.g., precipitation of Candida, *E. coli*, and BCG and *M. avium* antibodies by Streptococcus type A – but not type B – antibody) have no obvious implications for AIDS specifically, the majority of the 75 positive results involved immunological interactions between specific pairs of infectious agents associated with AIDS and between these antibodies and antibodies against lymphocyte antigens (HIV + Mycoplasmas; CMV + Mycoplasmas; HIV + Staphylococcus; and HBV + Mycobacteria, all of which also precipitated at least one lymphocyte antibody as well). These results suggest that sperm-associated antigens are not the only probable causes of autoimmune phenomena in AIDS – a crucial point that must be borne in mind in interpreting the data that follow.

All but one of the 22 positive results of the 454 DAD experiments relevant to sperm antigens and Mycoplasma antigens which were observed are shown in Figs. 3–9. All combinations not mentioned in the following paragraphs (that is a total of 139 antibody combinations with sperm antibodies and 283 with Mycoplasma and Mycobacteria antibodies) were repeatedly negative (no precipitation was visible).

Sperm immune serum raised in sheep against human sperm (Chemicon) precipitated antibodies against blood coagulation factors V, IX, and von Willibrand's factor; platelet glycoprotein beta (see Table 2); natural killer cells and follicular dendritic cells; and *Mycoplasma fermentans* (both Tully – not shown – and Lo strains) (Figs. 3 & 4). Sperm immune serum (Arnel) had more limited, but similar, specificities, precipitating antibodies against blood coagulation factor V (Fig. 5) *Mycobacterium avium* (Fig. 6), and *Mycoplasma fermentans* (Tully) and *Mycoplasma pirum* (Tully) (Fig. 7). These results strongly suggest that autoimmunity against blood coagulation factor V may result from immunological exposure to an appropriate sperm-cofactor combination, and that both Mycoplasma and Mycobacteria coinfections may play the role of cofactors for sperm when coprocessed by the immune system. Extensive evidence has previously linked Mycoplasmas and Mycobacteria to many autoimmune processes, including antisperm antibody-mediated forms (Soffer *et al.*, 1990; Shoenfeld & Isenberg, 1988). Furthermore, the coprecipitation of sperm and Mycoplasma antibodies confirms the chemical complementarity suggested by earlier research showing that Mycoplasmas can bind to sperm (Black, 1983). A similar mechanism may be involved in the transmission of *M. avium*.

We also found that *Mycoplasma fermentans* antibodies will precipitate a wide range of anti-HLA class II protein and macrophage antibodies (Figs. 8 & 9). These findings, combined with the precipitation of natural killer cell and follicular dendritic cell antibodies by sperm antibodies, confirm our theoretical predictions that a combination of sperm and Mycoplasma can induce idiotype/anti-idiotype antibodies that cross-react with determinants on cells of both CD4 and class II MHC lineages (see Fig. 2). Antibody to *Mycobacterium avium* did not precipitate any human lymphocyte antibody tested (a total of 14) and therefore

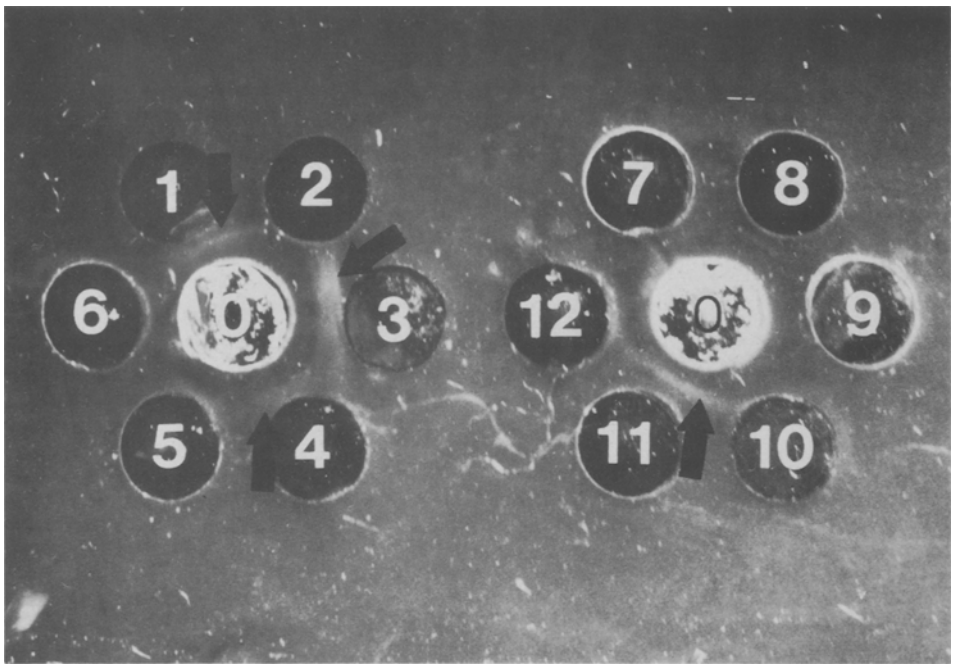

Fig. 3. Double antibody diffusion (DAD) study of sperm polyclonal Ab (Chemicon) with various human blood protein antibodies. Precipitations occurred between the sperm Ab (well 0) and antibodies against factor IX (well 1), factor V (well 3), follicular dendritic cells (well 4), and platelet glycoprotein 1 beta (well 11). Unstained. These precipitates model the immune complexes predicted in Figures 1 and 2.

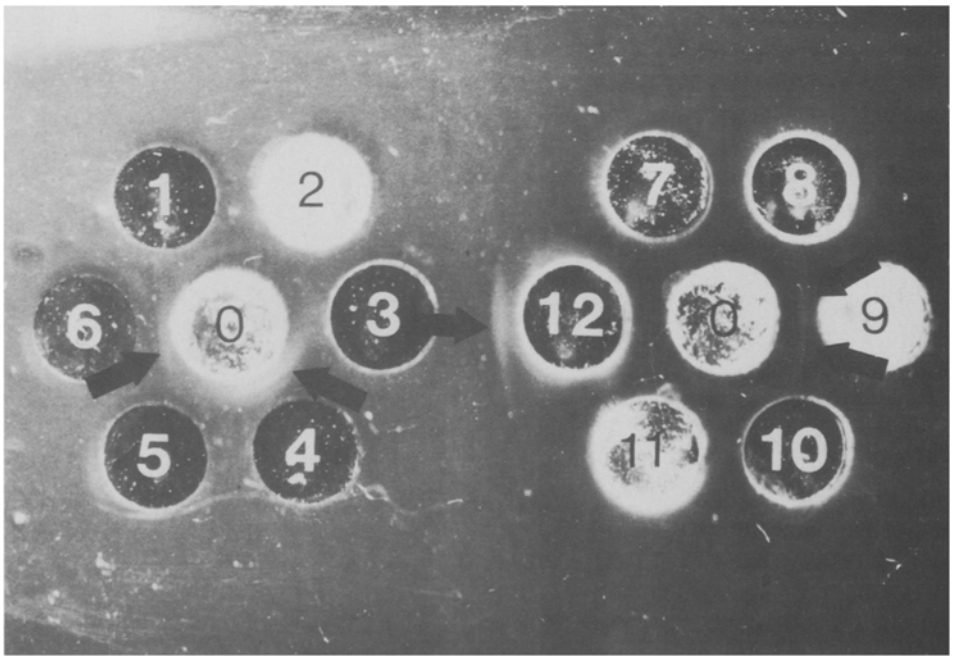

Fig. 4. DAD study of sperm polyclonal Ab (Chemicon) with antibodies to various blood proteins and infectious agents associated with AIDS. Precipitations occurred between the sperm Ab (well 0) and antibodies against von Willibrand's factor (well 4), natural killer cells (well 6) and *Mycoplasma fermentans*, strain *incognitus* (polyclonal; well 9). An additional precipitation occurred between Ab to *Mycoplasma hyorhinus* (well 12) and Ab to HLA DR (well 3). Mycoplasma-HLA complementarity is common – see Figs. 8 and 9. Unstained. These precipitates model the immune complexes predicted in Figs. 1 and 2.

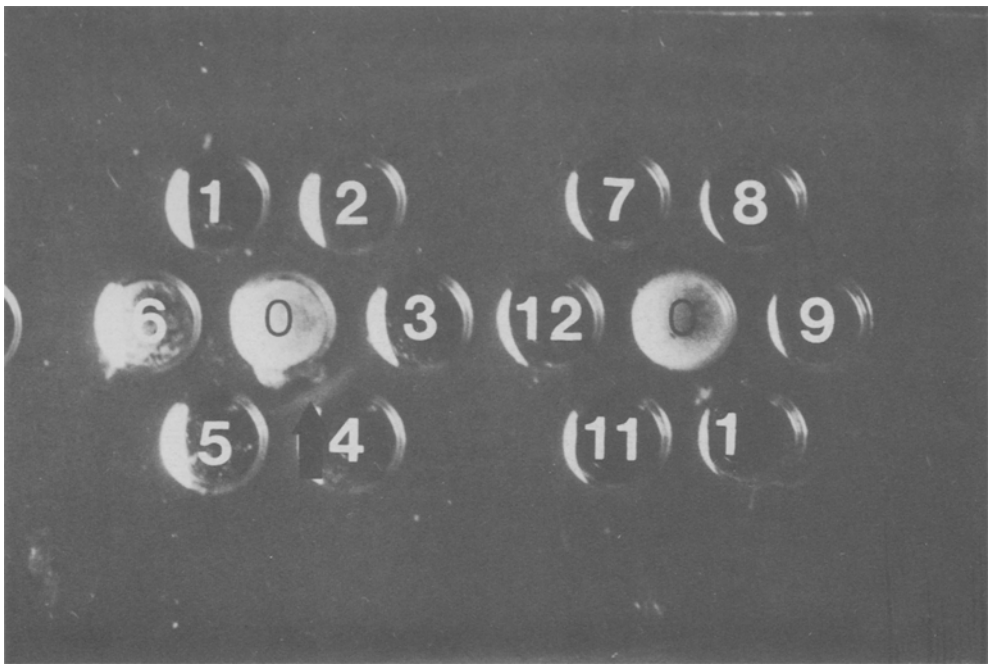

Fig. 5. DAD study of sperm polyclonal Ab (Arnel) with various antibodies to blood proteins and viruses. The only observed precipitation occurred between sperm Ab (well 0) and Ab to Factor V (well 4). Unstained. These precipitates model the immune complexes predicted in Figs. 1 and 2.

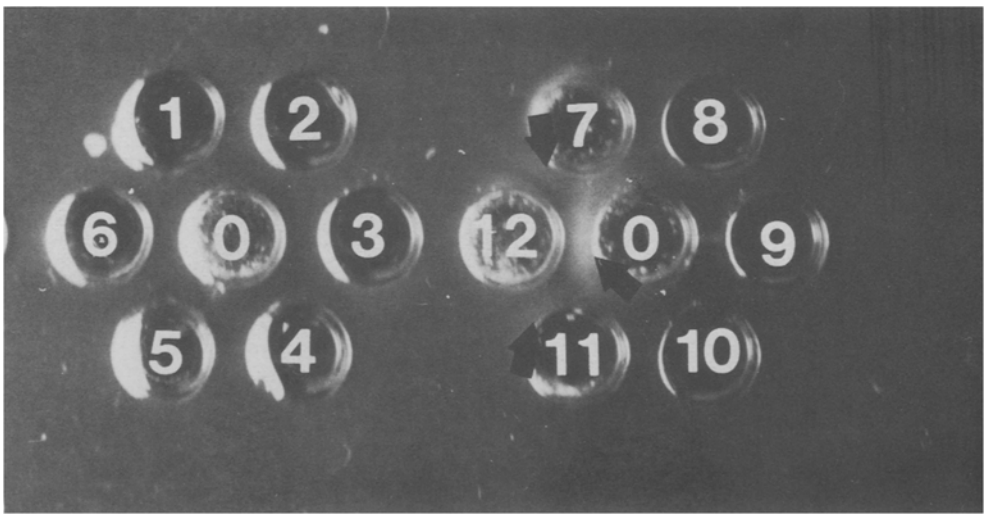

Fig. 6. DAD study of sperm polyclonal Ab (Arnel) with various antibodies to infectious agents associated with AIDS. The only observed precipitation was between sperm Ab (well 0) and antibody to *Mycobacterium avium* (well 12). Other Mycobacteria did not precipitate sperm Ab (data not shown). Very weak precipitations also occurred between *M. avium* Ab (well 12) and *S. aureus* Ab (well 7) and Streptococcus type A Ab (well 11) – see text for discussion. Unstained. These precipitates model the immune complexes predicted in Figs. 1 and 2.

appears to have different specificities than Mycoplasma antibodies. We also found no HIV antibody that precipitated either sperm antibody tested, although this negative result may be due to choice of antibodies tested rather than a true failure of sperm and HIV antibodies to cross-react (as would be predicted by experiments reported by other investigators in the Introduction, above).

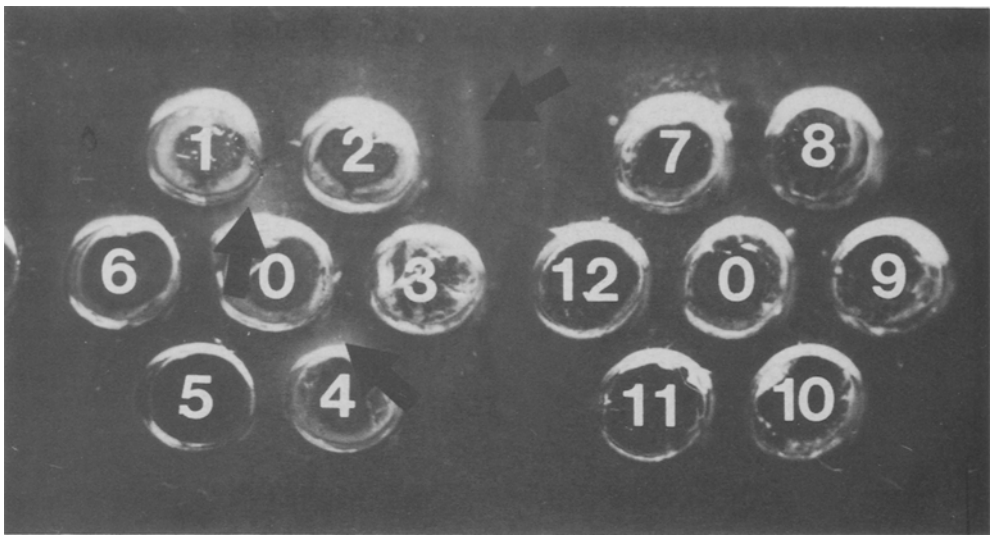

Fig. 7. DAD study of polyclonal Ab to sperm (Arnel) with antibodies to Mycoplasma and Mycobacteria. Precipitations occurred only between sperm antibody (well 0) and antibody to *M. pirum* (well 1) and *M. fermentans* (well 4). *M. hominus* antibody (well 2) and *M. pneumoniae* antibody (well 7) also precipitated each other, an observation without obvious relevance to the theory being tested. Unstained. These precipitates model the immune complexes predicted in Figs. 1 and 2.

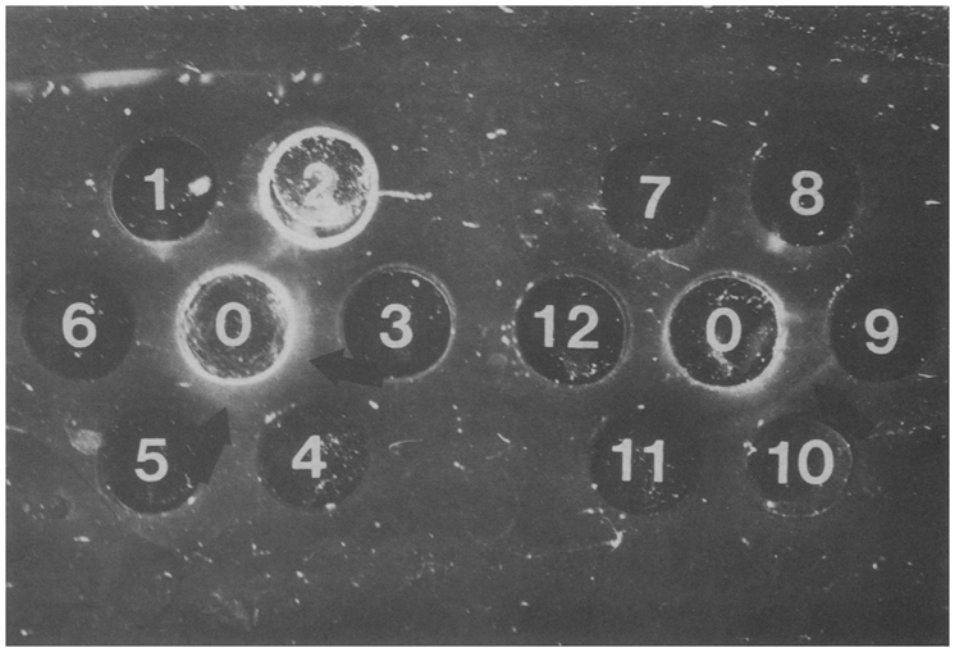

Fig. 8. DAD study of polyclonal Ab to *M. fermentans* strain *incognitus* (Lo) with antibodies to human blood proteins and infectious agents associated with AIDS. Precipitations occurred between *M. fermentans* Ab (well 0) and HLA DR common MAb (well 3), macrophage polyclonal Ab (well 5) and HBV surface protein polyclonal Ab (well 10). Unstained. These precipitates model the immune complexes predicted in Figs. 1 and 2.

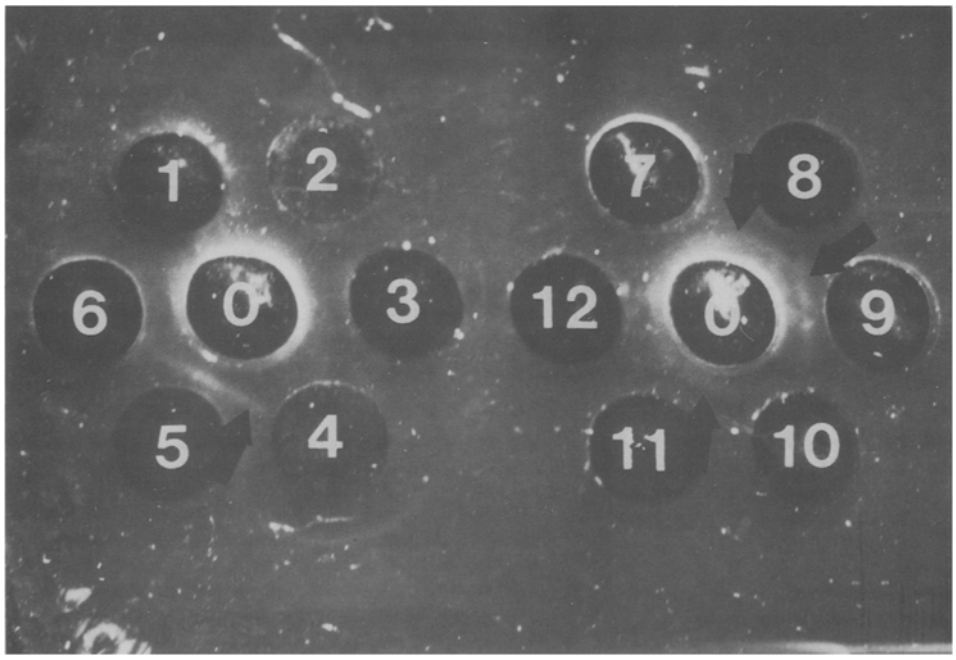

Fig. 9. DAD study of *M. fermentans* polyclonal Ab (rabbit; Tully) with blood protein antibodies. Precipitations occurred between Ab to *M. fermentans* and HLA-2 polyclonal Ab (well 5), HLA DQw1 MAb (well 8), HLA DQw52 MAb (well 9) and platelet glycoprotein 1 beta MAb (well 11). Unstained. These precipitates model the immune complexes predicted in Figs. 1 and 2.

The strong co-precipitation of sperm, platelet, and factor V antibodies is particularly noteworthy, since a relatively common symptom of AIDS is a blood coagulation problem that is not treatable by factors VIII or IX (Morrow *et al.*, 1991). Our data suggest that treatment with factor V might be beneficial. Also noteworthy is the possibility that the blood coagulation disorder is not due to HIV, but rather to sperm-induced autoimmunity, since inoculation of semen into animals is also associated with blood coagulation disorders and cellular fragility (Favilli, 1931; Metalnikoff, 1900).

Also of possible interest is the precipitation of *M. fermentans* antibody of antibody to HBV surface protein (Fig. 9). This observation suggests that HBV and Mycoplasmas may act synergistically in people who are co-infected, and have the potential to trigger autoimmune processes.

We must emphasize that these experiments are not a definitive examination of the possible cross-reactions that sperm and sperm-associated antibodies may have. The number of cellular and pathogen-related antibodies tested was limited by funds and availability. Thus, these experiments should be interpreted in terms of the hypothesis being tested, from which point of view they were successful. On the other hand, further testing may reveal that HIV or other infectious agents associated with AIDS may be able to act as cofactors with sperm to induce either anti-lymphocyte autoimmunity or other forms of autoimmunity associated with AIDS.

We must also emphasize that these experiments are, as far as we know, the first to demonstrate that independent antigens can result in pairs of idiotypic antibodies that act like idiotype/anti-idiotype pairs. All previous experiments describing idiotype/anti-idiotype antibody pairs have raised the anti-idiotype antibody from the idiotype.

Discussion

Autoimmune risks associated with receptive anal intercourse

The experimental data presented here, in conjunction with the extensive literature review documenting the presence of antibody responses to semen antigens and LCTAs in AIDS, strongly suggest that semen antigens may play a pathogenic role in AIDS, when processed by the immune system in the presence of appropriate infectious or allogeneic cofactors. Sperm has previously been shown to have CD4-like proteins on its cell sur-

face, and our similarity searching has revealed a wide range of possible sequences that may represent these CD4-like regions. Verification that any one of these sequences is, in fact, CD4-like in its antigenic potential clearly will require further experimentation. We have additionally demonstrated that some of the infectious agents associated with AIDS induce antibodies that are chemically complementary to those raised against sperm, and that some of these antibodies also precipitate antibodies against MHC class II proteins. These data suggest that some of the combination of agents, such as HIV plus semen, or HIV plus Mycoplasmas, that are predicted by the similarity searching to be chemically complementary pairs, are in fact so, and the data demonstrate that these pairs are cross-reactive with lymphocyte antigens. Thus, one unique prediction of the MAMA theory of autoimmunity is satisfied and its plausibility as a mechanism for inducing autoimmunity bolstered. We caution that the theory is far from proven. (Such proof will require inducing autoimmunity in animals using appropriate antigen pairs.) We also caution that these data do not address the question of whether alloantigen-induced autoimmunity is a primary cause of immune suppression in AIDS, a cofactor, or a symptom. The data simply show that our understanding of some of the mechanisms underlying the autoimmunity are a bit more comprehensible than before, and provide a specific basis for testing animal models based upon the specific pairs of antigens described here. If such animal models can be developed for these and other antigen pairs described in our other papers, then it will be possible to determine the extent to which they play primary or secondary pathogenic roles in AIDS. It will also be possible to begin developing prophylactic and treatment regimens appropriate to each autoimmune condition.

An important point needs to be stressed in interpreting both the protein similarity and antibody binding data just presented. The results of such autoimmunity may not be limited to HIV-infected individuals. Heterosexual women, and homosexual and bisexual men who engage in unprotected receptive anal intercourse, may have immunological contact with semen in the absence of an HIV infection. Such exposure, *concomitant with an appropriate antigenic co-factor* such as a simultaneous Mycoplasma infection, would be expected to induce anti-sperm antibodies that could act as lymphocytotoxic autoantibodies (LCTAs). One would therefore expect to find such autoantibodies accompanied by immunosuppression in many homosexual men (many because of the high rates of STDs in gay men) and some women (some, because of the relatively low rate of STDs compared with gay men) who practice receptive anal intercourse, even in the absence of HIV infection. This is, in fact, the case.

Kiprov and Anderson (1985) found LCTAs in both HIV-positive and HIV-negative homosexuals. Pruzanski, Jacobs and Laing (1983) found that all of their homosexual patients with AIDS or ARC had LCTAs against both T and B cells, but so did 13 of 17 symptom-free homosexual men. It is unlikely that all 13 were HIV-infected during 1982, when this study was conducted. Kloster, Tomar, and Spira (1984) reported similar results. Nine of ten AIDS patients had LCTAs, ten healthy heterosexuals did not, while three of five healthy homosexual men also had evidence of LCTAs. Further tests were done on two of these three men, and both showed significantly depressed T-cell activity and counts. Cleghorn *et al.* (1988) also report that T-cell counts and relative ratios were depressed to an equal degree in healthy homosexual men in Trinidad regardless of HIV status. Unfortunately, they did not test for the presence of LCTAs. Neither did Murray *et al.* (1988), but they too found that T-helper cell counts and activity were nearly identical when asymptomatic HIV-negative homosexuals were compared with asymptomatic HIV-positive homosexuals, but both groups were immunosuppressed compared with controls. In short, these data all suggest that HIV is neither necessary nor sufficient to cause T-cell depletion and immunological disfunction in homosexual men, and that other causes of such abnormalities, such as LCTAs induced by immunologic exposure to allogeneic cells, may be essential to the acquisition of HIV infection and for the subsequent transition from HIV-positivity to AIDS by creating prior immune suppression.

It is important to emphasize that the risk of developing anti-sperm induced antibodies that also act as LCTAs is not limited to homosexual men. Many heterosexual couples engage in anal intercourse. The anus is unlike the vagina in several significant ways: it does not have a thick layer of epithelial cells or mucus producing cells to protect it; it lacks the musculature of the vagina; and the vasculature is much closer to the surface. The lower intestine is also studded with Peyer's patches, which serve as immunological portals for sampling the intestinal contents. All of these factors make immunological contact with semen components much more likely in anal than in vaginal sex. Thus, it is significant to note that a Kinsey Institute study (Anonymous, 1988) recently revealed that between 30

and 40% of all American women over the past four decades have experimented with anal intercourse at some time during their lives, and that as many as 25% of sexually active women at one midwestern university had engaged in anal intercourse at least once during their college years. These figures are similar to those reported for university students in Canada (MacDonald *et al.*, 1990). During 1988, 5514 first year college students were surveyed. Of the 69% of the women who were sexually active, 19% had participated in anal intercourse at least once, and 5.5% reported a previous sexually transmitted disease. Among the 8.6% of the women who had had ten or more partners, however, 35% had engaged in anal intercourse, and 24% had had one or more STDs. These data are relevant to understanding risk for immune suppression, since several studies have demonstrated that both heterosexual anal intercourse and STD history confer the same increased risk of AIDS in women as has been found among homosexual men. We stress again, however, that simply engaging in unprotected receptive anal intercourse does not necessarily lead to antigenic exposure to semen, nor to exposure to the specific combinations of antigens that we believe to be necessary to induce LCTAs. The risk is also a function of what infectious agents are present in the semen, or in the anus, at the time of exposure, as well as the number of partners (since the greater the number of partners, the greater the range of alloantigen challenge and the greater the probability of an appropriate antigenic cofactor being present at the time of exposure).

Unprotected receptive anal intercourse has also been reported to be a significant AIDS risk for spouses of hemophiliacs (Melbye *et al.*, 1985). The European Study Group (1989) reports that a study of 153 HIV-seropositive men and their wives resulted in the identification of three factors that predisposed to acquisition of HIV infection: a history of one or more sexually transmitted diseases in the woman during the past five years; full-blown AIDS in the male partner; and anal intercourse (note the combination of STD with anal intercourse, which is required by the MAMA theory). Seropositivity among women with none of these risk factors was 7%, whereas it was 67% among women having two or more risk factors. Padian, Shiboski and Jewell (1990) have reported similarly that the two highest risk factors for heterosexual transmission of AIDS are repeated anal intercourse and the occurrence of bleeding during intercourse. These results seem to be confirmed by a study of 1115 women who attended a genitourinary medicine clinic in west London during 1987. Three of the women were HIV seropositive. Two of these women completed a questionnaire concerning their sexual history. Both women were in the upper fifth percentile for lifetime sexual partners, and both were unusual in having engaged repeatedly in anal intercourse (Evans *et al.*, 1988). Moreover, all of the women infected by a male hemophiliac in Great Britain were found to have engaged in repeated, unprotected anal intercourse with him (Maddox, 1992; Hodgkinson, 1992) and anal intercourse has been reported to be a risk in many other hemophiliac-associated AIDS cases (Melbye *et al.*, 1985). Unfortunately, immune competence has not been measured in any of these studies, nor has any attempt been made to determine if anti-sperm antibodies, lymphocytotoxic antibodies, or circulating immune complexes are also associated with anal intercourse as might be expected according to the data reported above. It would seem worthwhile to carry out such studies since the degree to which semen-associated autoimmunity may predispose or contribute to AIDS is clearly of importance.

Acquired immunosuppression unassociated with HIV can be sufficient to lead to opportunistic infection. Cases of opportunistic infections including *Mycobacterium avium intracellulare*, esophageal candidiasis and Kaposi's sarcoma have been reported repeatedly in HIV-negative homosexual men and drug addicts (reviewed in Root-Bernstein, 1990a; 1993) and many hundreds of HIV-free AIDS cases – now subsumed under the name idiopathic CD4-T-cell lymphophenia (ICL) – have now been recorded by the Centers for Disease Control (Atlanta, GA, USA). For example, recently Macon *et al.* (1993) have characterized a homosexual male patient with Kaposi's sarcoma and atypical lymphoma without signs of HIV or HTLV-I or II infection who developed Pneumocystis pneumonia and died of disseminated *Cryptococcus neoformans* infection. His T-cell count at diagnosis was only $43/mm^3$. He was, however, infected with *Mycoplasma fermentans* – a clearly relevant finding in light of the results reported here (see also Soffer *et al.*, 1990). Similarly, Tsatsoulis and Shalet (1991) have characterized a 26 year old man with polyglandular autoimmune (PGA) syndrome, characterized by Addison's disease, hypoparathyroidism, chronic mucocutaneous candidiasis, and alopecia totalis (all of which are symptoms of T-cell suppression and are also common features of AIDS). He also had extremely high levels of sperm antibody, which could have been a very likely cause, or at least a significant mediator, of his clinically-apparent immune suppression.

It is important to stress that the results obtained in our experiments strongly support the work of Montagnier and Lo concerning a primary pathogenic role for Mycoplasmas in AIDS etiology (Montagnier *et al.*, 1990; Lo *et al.*, 1991). At the same time, we stress that Mycoplasmas are only a part of a larger pathogenic picture that includes immunologic exposure to semen components, allogeneic lymphocytes, HIV, CMV and other infectious agents, and that it is too simplistic to attribute AIDS pathology to any single agent or mechanism. We therefore interpret our results conservatively to mean only that sperm-associated autoimmune processes can play a role in AIDS, but not that they are a necessary or sufficient factor for AIDS causation.

Thus, semen-induced autoimmunity directed at lymphocytes may be a cause of immune suppression independent of HIV infection (though it is certainly not the only cause) (Root-Bernstein, 1990a, 1990b, 1990c; 1993); it may predispose individuals to contracting an HIV infection or it may act as a co-factor or stimulus to HIV replication that contributes to the development of full-blown AIDS following infection. At this point in time, existing data do not allow us to choose between these alternatives. We therefore suggest that prospective studies of the immunological status of individuals who engage in frequent or promiscuous unprotected anal intercourse both in the presence and absence of HIV infection would be of great importance to our understanding of the etiology and pathogenesis of AIDS, and perhaps lead to greater understanding of the immune suppression that often precedes HIV infection in high risk individuals.

Further epidemiologic and experimental tests of the role of autoimmunity in AIDS and ICL

A number of testable issues need to be addressed concerning the possible roles of lymphocyte and sperm antigens as inducers of pathologic autoimmune processes in AIDS and ICL. First, we are not claiming that such autoimmunity is *the cause* of either syndrome. Rather, we are making the much more limited claim that these agents may be inducing clinically relevant pathological events that may range from severely immune suppressive to merely additive immunological burdens. Thus, we have two responses to the common argument that if semen were a 'cause' of AIDS, AIDS would be as old as mankind, since homosexuality is undoubtedly as old as mankind. Our first response is that we are not claiming that exposure to semen is sufficient to cause AIDS. That is something that needs to be elucidated by experiment. More importantly, people who make the 'homosexuality-is-as-old-as-mankind' argument ignore two limitations to our 'epidemiological' evidence from the past.

First, the diseases that are diagnostic for AIDS in the absence of T-cell tests and HIV tests are all extremely difficult to diagnose, and all of them (save Kaposi's sarcoma and disseminated tuberculosis) were discovered and became readily diagnosable only in the past 30 to 40 years (Root-Bernstein, 1993). Furthermore, prior to the antibiotic era, people who developed severe immune suppression would have been more likely to die of much more common diseases, such as syphilis, tuberculosis, or sepsis, than of the rare opportunitistic infections we see today (Root-Bernstein, 1993). Thus, prior to the antibiotic era, it would have been extremely difficult to identify AIDS, even if it existed, although hundreds of cases meeting the surveillance definition of AIDS are known back to 1872 (Root-Bernstein, 1990c; 1993).

Also to the point, homosexual behaviors have changed radically over the centuries. Upper class gay men in Greece and Rome, for example, were forbidden to allow themselves to be entered sexually. Anal intercourse was practiced only on social inferiors (Aries & Duby, 1987). In other words, socially superior men 2000 years ago would have had very low probabilities of contracting AIDS, whatever the cause or causes of AIDS. Since most of our medical records from antiquity are by and about socially superior men, and since these medical men did not have the disease categories necessary to observe AIDS-like symptoms, they are unlikely to record AIDS-like symptoms even if they had been present. In short, historical evidence is largely irrelevant to the present question.

The present questions are of mechanism and pathogenesis: What causes lymphocytotoxic autoimmunity in AIDS, and what role does this autoimmunity play in AIDS? We want to stress that sperm-induced autoimmunity, like may other forms of autoimmunity (Westall & Root-Bernstein, 1986; Root-Bernstein, 1990b, 1991) probably either requires or can be promoted by infectious co-factors such as HIV, cytomegalovirus, the various hepatitis viruses, chlamydia, and other sexually transmissible agents (Root-Bernstein, 1990b; Root-Bernstein & Hobbs, 1993). Therefore, we are not arguing that immunological exposure to sperm by itself will necessarily lead to lymphocytotoxic autoimmunity. There are certainly many reports of AIDS-associated diseases being transmitted by anal intercourse in the absence of HIV both in homosexual men (Morson,

1964; Felman & Nikitas, 1979; Turner, White & Soutter, 1990; Buimovici-Klein *et al.*, 1990) and in women (Rabinowitz, Bassan & Robinson, 1988). The importance of these infectious agents as possible co-factors in inducing autoimmunity is emphasized by the observation of numerous homologies between CMV, EBV, HSV, HBV and other infectious agents and the CD4 protein of T-helper cells (Root-Bernstein & Hobbs, 1993).

Given the large amount of data demonstrating many similarities between semen-associated components and lymphocyte proteins, and the widely validated role of lymphocytotoxic autoimmune process in AIDS, it now becomes manifestly important to perform new types of animal experiments (Root-Bernstein, 1990c, 1992b, 1993). The role of semen-Mycoplasma, HIV-semen, or HIV-semen-Mycoplasma synergism could be evaluated by inoculating chimpanzees or macaques rectally with these combinations and evaluating both the production of LCTAs and the rate of development of immune deficiency as compared with animals inoculated with single agents. Similar experiments could be performed to evaluate allogeneic lymphocyte-Mycoplasma, allogeneic lymphocyte-HIV, and allogeneic lymphocyte-HBV (or other viruses) effects when inoculated intravenously, as occurs in drug abuse and transfusion. Finally, many of these same experiments can be performed on non-primate models to determine whether actual HIV *infection* is necessary for development of LCTAs and immunosuppression, or if simple antigenic stimulation (i.e., production of antibody) with appropriate pairs of transmissible agents may be sufficient to trigger autoimmunity.

The importance of experiments to model the induction of autoimmunity in AIDS and ICL cannot be overestimated. No animal infected with HIV has ever displayed evidence of LCTAs or CICs (Clerici *et al.*, 1993; Zarling *et al.*, 1990; Stricker *et al.*, 1987b). No animal infected with HIV has ever developed AIDS, either. On the other hand, development of LCTAs and CICs in human beings at risk for AIDS and ICL is as highly prognostic of death as any HIV-related measure of health, whereas absence of LCTAs and CICs is highly predictive of continued health and normal T-cell counts, even among HIV-infected people (Szabo *et al.*, 1992; Zarling *et al.*, 1990; Sonnabend, 1989; Ozturk *et al.*, 1987; Stricker *et al.*, 1987a; 1987b; McDougal *et al.*, 1985). These observations strongly support the contention that autoimmune phenomena are central to AIDS (as opposed to HIV infection), whether their mechanism(s) of induction turn out to be due to semen components, allogeneic lymphocyte exposure, multiple concurrent infections, or some combination(s) of these (Root-Bernstein, 1990c, 1993; Root-Bernstein & Hobbs, 1993). We are not dogmatic about the possible causes of autoimmunity in AIDS – only that we must find its causes, whatever they turn out to be.

Moreover, we firmly believe that there can be no cure for AIDS without a concomitant cure for the autoimmune phenomena that plague all people with AIDS, for it is the nature of autoimmune processes to progress independently of the agents that initiate them (Root-Bernstein, 1991, 1993). No greater calamity could befall AIDS research than to learn how to control HIV, but still have AIDS patients die of uncontrolled autoimmune processes, or to have people return to high risk behaviors, thinking that they are safe because they cannot get HIV, only to find that there are causes of autoimmune destruction of the immune system that do not require HIV.

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