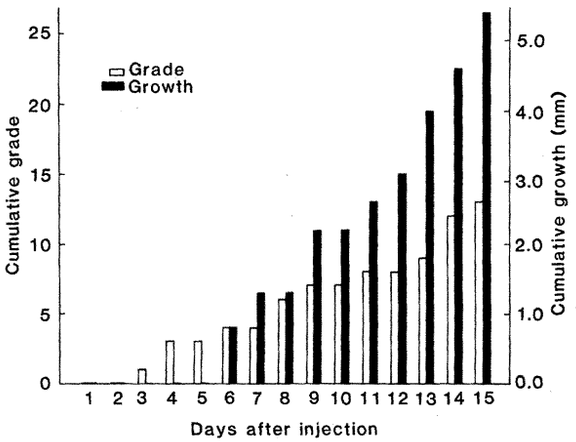

Fig. 2. Cumulative growth of new blood vessels after injection of hFF into rabbit corneas (N = 8).

4, vessels extending more than 2.0 mm; 5, vessels extending more than 3.0 mm; and 6, vessels extending more than 4.0 mm. Measurements of vessel growth in millimeters per day were also obtained. Eyes that demonstrated angiogenesis were removed, fixed, and stained with hematoxylin and eosin for microscopic evaluation.

Undiluted hFF stimulated angiogenesis in eight of eight rabbits (Fig. 2). The invasion of blood vessels was macroscopically evident 3 days after injection and by day 15 extended 2.0 mm into the injection site from the closest part of the corneal-scleral limbus. Two- and four-fold dilutions of hFF stimulated angiogenesis 5 and 3 days after injection, respectively, in two of four rabbits, with the vessels seen at 3 days extending 1.0 mm into the injection site by day 15.

Dialyzed hFF induced angiogenesis in two of three rabbits, with vessels extending up to 1.0 mm into the injection site by day 15. Charcoal-treated hFF produced angiogenesis in three of three rabbits, with a plexus of blood vessels growing more than 4.0 mm by day 15. In contrast, intracorneal injection of heated hFF did not result in corneal vascularization.

None of 20 control rabbits injected with Hydron and saline and three rabbits treated with hCG and Hydron showed any angiogenic activity. Histological examination of sections prepared 3 weeks after the injections did not reveal any foreign bodies or marked inflammatory reaction in the injection site.

During the follicular phase of the human menstrual cycle a single follicle usually matures and ovulates in response to the midcycle surge of serum gonadotropins. The question arises as to why only a single follicle develops to maturity while others undergo atresia in the same gonadotropin environment.

Basset (*2*) described the appearance of perifollicular blood vessels during growth and regression of rat ovarian follicles. From the endothelial walls of these vessels angiogenic sprouts began to grow into the granulosa layer, and this was associated with a rapid increase in capillary permeability. Jakob *et al.* (*3*) showed that the rat corpus luteum produced a vigorous stimulation of capillary growth. Koos and LeMaire (*4*) described the induction by rat follicles and corpora lutea of gonadotropin-responsive angiogenesis in the chorioallantoic membrane of the chick embryo. Gospodarowicz and Thakral (*5*) found that explants of rabbit corpora lutea stimulated angiogenesis when placed into a rabbit cornea. Taken together, these results suggest that a signal for angiogenesis accompanies follicular development and formation of the corpus luteum in these species. Recent evidence by diZerega and Hodgen (*6*) and Zeleznik *et al.* (*7*) demonstrated an increasing density of blood vessels in the complex of the dominant follicle in primates. This increase in perifollicular vascularization may result in preferential gonadotropin delivery and thus in selection and maintenance of the dominant follicle.

The cornea provides a naturally transparent, avascular substratum in which neovascularization can be continuously monitored (*8, 9*). Using the corneal implant assay we found that hFF contains an angiogenic factor that may be involved in neovascularization. Three days after injection of hFF, new blood vessels were visible; by day 6 these extended more than 1 mm into the cornea. Thereafter vessel growth continued progressively. We conclude that developing follicles produce an angiogenic factor and that this selective vascularization may be important during preovulatory folliculogenesis.

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Rectal Insemination Modifies Immune Responses in Rabbits

Abstract. *Weekly deposition of pooled rabbit semen into the rectum in healthy male rabbits resulted in the appearance of immune complexes and antibodies to sperm and to peripheral blood lymphocyte antigens. It also led to a decreased ability to mount a humoral immune response to T lymphocyte–dependent antigens, keyhole limpet hemocyanin, and sheep red blood cells. These findings suggest that repeated rectal deposition of semen may compromise some aspects of the immune system.*

Antibodies to spermatozoa and to circulating immune complexes (CIC's) are present in apparently healthy homosexual men (*1, 2*) and at increased concentrations in homosexuals with lymphadenopathy or acquired immunodeficiency syndrome (AIDS) (*2, 3*). The etiology of AIDS is not clear; however, the fact that a large proportion of AIDS cases have been found in highly sexually active homosexual men suggests that the syndrome may have some relation to circulating antibodies evoked as a result of semen deposition in the alimentary canal. Human seminal fluid apparently contains components that potentially can suppress the immune response (*4, 5*), and syngeneic mouse spermatozoa in-

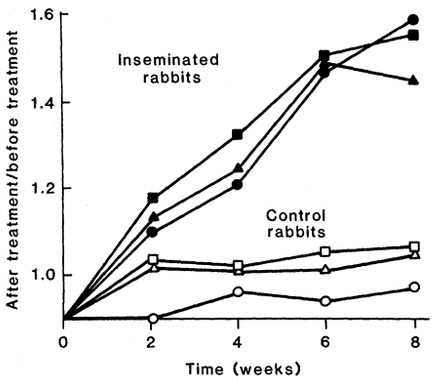

Fig. 1. Appearance of CIC's and sperm-reactive IgG and IgA after rectal insemination. Plasma from five inseminated rabbits and five controls were assayed for CIC's by a Raji cell ELISA (*16*) and for IgG and IgA by an ELISA with rabbit sperm bound to wells of a microtiter plate (*1*). Each value is the mean of at least three determinations that varied less than 10 percent. Symbols: (●, ○) IgG, (▲, △) IgA, and (■, □) CIC's.

jected intravenously are reported to be immunosuppressive as determined by mixed lymphocyte culture (*6*).

We used rabbits to ascertain whether CIC's or antibodies to sperm can result from rectal insemination and whether this affects the ability to mount an immune response. Rabbit ejaculates collected with an artificial vagina were pooled and examined with a microscope for motile sperm, which were counted. Healthy males were restrained and 1 ml of fresh semen (containing about 10^8 sperm) was deposited in the rectum to a depth of 5 cm at weekly intervals with a No. 7 French rubber catheter. To ensure that this had not traumatized the rectal mucosa, feces passed after the procedure were examined and found to be heme-negative. Rabbits treated by depositing 1 ml of saline in the same manner on the same schedule were used as controls.

Blood was drawn into heparinized tubes at weekly intervals for immunological analyses. The total red and white cell counts, differential white cell count, and total concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) in the serum were not affected by these treatments. Nor was the ability of concanavalin A, phytohemagglutinin, and rabbit spermatozoa to induce proliferation of the peripheral blood lymphocytes (PBL's) reduced. However, after an 8-week period of weekly rectal inseminations there was a significant ($P < 0.01$, Student's *t*-test) increase in CIC's and in IgG and IgA reactive with rabbit sperm (Fig. 1); no changes were seen in the control rabbits. Antibodies to seminal fluid antigens were not detected.

Rectal insemination for longer periods (up to 8 months) did not bring about a further increase in CIC's or antibodies to sperm. However, levels of IgG reactive with normal rabbit PBL's, apparent by 2 to 4 months, increased throughout the 8-month period. Serum samples obtained from each rabbit before treatment and at the end of treatment were assayed for IgG that reacted with normal rabbit PBL's. Immunoglobulin G reactive with PBL's in semen-treated rabbits doubled on average, whereas saline-treated controls showed no such change ($P < 0.01$, Student's *t*-test) (Table 1).

In addition to producing IgG reactive with PBL's, semen-treated rabbits produced IgG reactive with asialo GM1 (*7*). This neutral glycolipid is not present on B cells (*8*) but occurs on the surface of murine natural killer and peripheral T cells and on rabbit and human sperm (*7*). Previous studies have shown that injection of antibody to asialo GM1 into mice (*9*) or rats (*10*) decreases natural killer cell function in these animals and that 36 percent of homosexuals with AIDS have antibody to asialo GM1 in their serum (*7*).

We also studied the responsiveness of semen-treated animals to T cell–dependent antigens—in this case keyhole limpet hemocyanin (KLH) and sheep red blood cells (SRBC's). Since the means for assessing natural killer cell function in rabbits have not yet been developed and since mitogen-induced lymphocyte proliferation appeared to be unaffected after rectal insemination, immune function in the experimental males was assessed by evaluating the immune response to these T cell–dependent antigens. Rabbits were immunized intravenously with KLH or SRBC's for 4 to 5 days and antibody responses were determined daily by measuring hemagglutination of KLH-coated or uncoated SRBC's (*11*) and by measuring KLH- or SRBC-specific IgG, IgM, and IgA with the enzyme-linked immunosorbent assay (ELISA) (*12*). Table 2 summarizes the hemagglutination results of SRBC-immunized normal and treated rabbits.

Table 1. Reactivity of IgG in serum from rectally inseminated rabbits with normal rabbit PBL's. The last serum sample from each rabbit and the sample obtained before treatment began were analyzed for IgG reactive with normal rabbit PBL's. The PBL's were prepared (*15*) and 5×10^4 cells were fixed with glutaraldehyde to the wells of microtiter plates. Wells were washed with phosphate-buffered saline (PBS) and Tween and incubated for 2 hours with 1:10 dilutions of test serum. IgG bound to the rabbit lymphocytes was then measured by incubating wells with 1:200 dilutions of alkaline phosphatase–conjugated swine antibodies to rabbit IgG (gamma chain–specific) for 90 minutes, washing wells with PBS-Tween, and adding 0.2 ml of *p*-nitrophenylphosphate (1 mg/ml) in 10 percent diethanolamine (*p*H 9.8). After 30 minutes the optical density at 405 nm was determined. Each value is the mean of at least three determinations that varied less than 10 percent.

Treatment	IgG (before treatment/after treatment)	Treatment	IgG (before treatment/after treatment)
Semen	3.4	Saline	0.9
Semen	1.1	Saline	1.1
Semen	2.0	Saline	1.2
Semen	2.8	Saline	0.7
Semen	1.7		
Mean	2.2		0.98

Table 2. Hemagglutination response to SRBC's by rabbits receiving rectal semen. Rabbits were given 2 ml of 10 percent SRBC's in 0.9 percent NaCl every day for 4 days. Before each injection and every day after the injections were discontinued, 3 ml of blood was collected for analysis of the SRBC hemagglutination titer (*11*).

Treatment	Day of peak titer	Peak titer
Saline	7	1:2048
Saline	6	1:2048
Saline	5	1:2048
Semen	6	1:64
Semen	6	1:2048
Semen	6	1:64
Semen	9	1:64

Table 3. Peak production of antibody to KLH in rabbits. Polystyrene microtiter plates were incubated with KLH (10 μg/ml) in carbonate buffer (*p*H 9.8) overnight at 4°C. The plates were washed in PBS-Tween and 0.1 ml of a 1:10,000 dilution of rabbit serum was added to each well. After being incubated at room temperature, the plates were washed with PBS-Tween and incubated with alkaline phosphatase–conjugated swine antibody to rabbit IgM, IgG, or IgA (heavy chain–specific) for 90 minutes. After a final wash with PBS-Tween, 0.2 ml of *p*-nitrophenylphosphate (1 mg/ml) in 10 percent diethanolamine (*p*H 9.8) was added to each well. The optical density at 405 nm was determined at 30 minutes and wells that had been reacted with normal rabbit serum were used as blank reference wells. Each value is the mean (and standard deviation) of at least three determinations for each of four rabbits.

Antibody	Optical density at 405 nm	
	Normal rabbits	Semen-treated rabbits
IgM	1.643 (0.216)	0.732 (0.110)
IgG	1.511 (0.124)	0.976 (0.201)
IgA	0.410 (0.116)	0.165 (0.086)

Hemagglutination in serum from rectally inseminated animals was significantly lower than that in serum from controls ($P < 0.01$), and IgM, IgG, and IgA titers were similarly diminished. Table 3 shows the outcome of a 5-day intravenous KLH immunization. A reduction in each antibody isotype was associated with animals receiving rectal semen. The differences in IgG, IgA, and IgM binding were all significant at $P < 0.01$, $P < 0.02$, and $P < 0.01$, respectively.

In conclusion, rectal insemination of homologous semen into rabbits had several consequences. Sperm in the rectum evoked the formation of antibodies to sperm despite the presence of seminal plasma, which has been held to be immunosuppressive (*5*). Furthermore, as judged by representative serial sections of tissue taken at autopsy, this response occurred in the absence of insemination-associated trauma to the gut lining or perianal area, possible elements in the etiology of the sperm antibody response seen in homosexual men (*1, 2*). While the rectally inseminated rabbits did not develop the opportunistic infections typically seen in AIDS patients, their reduced responsiveness to T cell–dependent antigens suggests a possible link in this respect. The basis of this effect is not yet known. We have found no significant difference in the rate of clearance of ^{51}Cr-labeled SRBC's in antigen-naïve inseminated and control rabbits, indicating that macrophage interaction with the antigen was not impaired after rectal insemination. Moreover, it is unlikely that the reduced humoral response was due to net lymphocyte depletion, since total PBL counts did not change. However, the appearance of CIC's and antibodies to asialo GM1 in the serum of rectally inseminated rabbits and the fact that serum from the rectally inseminated rabbits inhibited mitogen-induced proliferation of control PBL's in vitro (*13*) raises two possibilities. The first is that CIC's can interfere with immune function (*14*). They have been associated with a number of pathological states (*14*), including AIDS (*1, 2*). Second, the presence of IgG reactive with asialo GM1 in the serum of rectally inseminated rabbits may also be a factor in the decreased immune response. When presented in association with sperm-surface allogeneic histocompatibility antigens, this surface antigen may evoke an autoimmune response leading to the appearance of antibodies reactive with subsets of the T lymphocyte or natural killer cell populations. CIC's or cross-reactive antibodies could foster a degree of suppression by interfering directly with lymphocyte functions or by inducing alterations in the production of soluble mediators of the immune response.

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Location and Chemical Synthesis of a Pre-S Gene Coded Immunodominant Epitope of Hepatitis B Virus

Abstract. *Immunodominant, disulfide-bond independent epitopes recognized by human antibodies to hepatitis B virus (HBV) are located within the 55-residue amino terminal portion (coded for by the pre-S region of HBV DNA) of minor HBV envelope components larger than the major protein constituents encoded by the S gene. A peptide having the sequence of the first 26 amino acids from the amino terminal methionine was synthesized and elicited antibodies (at dilutions of ≥ 1 to 10^5) to the HBV envelope. These antibodies can be utilized for diagnostic tests. The immunogenicity of the peptide was substantially increased by covalent attachment to liposomes. The disulfide bond–independent determinants on sequences coded for by the pre-S gene may be more easily mimicked by peptide analogs than "conformational" determinants on the S-gene product.*

Hepatitis B virus (HBV) infections represent a major public health problem throughout the world. Available vaccines (*1*) produced from the serums of HBV carriers, because of limited resources and production costs involved, do not provide the appropriate means to control and eradicate the disease worldwide. Eventually this may be accomplished by vaccines based on either recombinant DNA technology or on synthetic peptides (*2–6*).

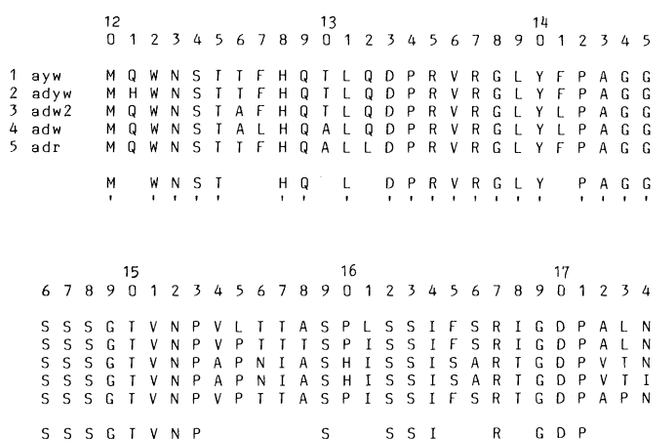

Fig. 1. Amino acid sequences of the translational products of the pre-S gene region downstream from Met 120 as deduced from sequences of HBV DNA (*21, 39–41*). The sequences are presented in one-letter-code words (*42*). Sequences for five distinct HBV subtypes are presented. Line 6 (bottom) shows amino acid residues common to all five subtypes. Single-letter abbreviations for the amino acid residues are A, alanine; R, arginine, N, asparagine; D, aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; F, phenylalanine; M, methionine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine.