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Development of an Immunocontraceptive Vaccine: Immunization of Female Baboons with Affinity Isolated Sperm Antigens

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Executive Summary

The purpose of the present project is to investigate the contraceptive potential of immunizing females with sperm proteins in order to generate an immune response which prevents with fertilization. This approach would maintain several improvements over conventional contraceptive methods: low cost, long period of efficacy, potential reversibility, minimum time commitment by both health care personal and patient, and wide spread access to the population.

In the course of this project we have utilized immunization schemes in baboons designed to generate a specific immune response in the female reproductive tract, where its effect on fertilization is desired. Tests to date do not indicate adverse systemic effects within the animal. Immunization techniques, and data analysis techniques have been established and optimized, and will be critical in the analysis of specific immune responses. Immune responses against whole sperm are below the level of detection. However, direct stimulation of the gut associated lymphoid tissue (GALT) with recombinant sperm antigen has resulted in detectable immune responses against sperm antigen.

Localization of immune cells (B and T cells) by immunohistochemistry indicates uneven localization within the reproductive tract. Distribution within the uterus is dependent on the stage of estrous cycle. Localization in the lamina propria vs

epithelin is also cycle dependent

Drs Nancy Alexander and John Herr have provided crucial intellectual and technical support, both in the experimental design and allotment of unique highly purified recombinant proteins unavailable from other sources.

Research Objectives

One of the fundamental causes of poor health in developing countries is the increasing population. Many of the current methods of contraception have proven ineffectual for a number of practical and cultural reasons including availability, side effects, education level, preference and cost. Immunocontraception refers to the use of vaccines which stimulate the immune system to prevent pregnancy. Individuals immunized with such a vaccine would be infertile for relatively long periods of time (1-3 years), such that it does not require large amounts of time, oversight, or experience on the part of either the patient or health care worker. Vaccines are also relatively inexpensive (possibly less than $1 per immunization). Immunocontraception would thus provide a noninvasive, inexpensive, long term form of contraception applicable in a number of underdeveloped areas.

One approach to immunocontraceptive vaccines is to immunize females against sperm antigens. This requires the generation of secretory immune responses in the female reproductive tract. The objective of the present project is to examine the possibility of generating such a secretory immune response in female baboons against baboon sperm antigens in order to interfere with fertilization. We carried out examination of humoral immune responses against sperm antigens immunized into the Peyer's patches of female baboons. The first objective was to examine whether this

immunization scheme would increase Ig levels within the reproductive tract. This required the establishment of Peyer's patch immunization protocols via laparotomy, collection of sera, vaginal and uterine flushes, and refinement of sandwich ELISA for Ig level determination. The second objective was to determine the specificity of the immune response against sperm antigens.

The results of this study are already being applied to other sperm antigen projects at the institute. There are 3 PhD students examining the efficacy of sperm antigens in prevention of fertilization. The present project has been carried out by Ms Hilda O'hara. Thus far she has exposed several pitfalls which can be avoided in the future, established techniques essential to the examination of secretory responses, and will generate antibodies against baboon Ig which will greatly facilitate the examination of immune responses against sperm as well as egg and parasite antigens. Mr. Peter Gichuhi is currently utilizing sperm antigens conjugated with cholera toxin to determine whether secretory responses can be induced with this method more efficiently than with PP immunization. Mr. Pius Adoyo is also exploring an alternate sperm antigen in these studies. Mr. Makokha is establishing sperm binding assays with which potential vaccines will be tested.

Several other organizations have funded immunocontraceptive development projects at IPR, on which this USAID grant has had a very positive impact.

Methods and Results

Part 1

Previous studies indicate that immunoglobulin levels vary during the estrous cycle, increasing in proestrus and estrus. Generalized stimulation of plasma cells at these points in the cycle could indicate an optimal time for immunization. Most of these studies have been carried out on immunized or infected animals, and not in normal cycling females. We therefore undertook to determine the normal Ig levels in cycling and non-cycling females. Stages of menstrual cycle were determined by visual observation using a point scale to quantify external sex skin swelling. Stages were confirmed by vaginal cytology. Sera and vaginal flushes were collected once each week for 6 weeks (one cycle) from 4 cycling adults and 2 non-cycling virgins under general anesthesia. Total protein in vaginal flushes was determined by Lowry assay, and standardized. Ig concentrations in sera and vaginal flushes were measured by ELISA using antibodies against human Ig. Sandwich ELISA of serum samples and flushes show higher levels of Ig during menses (Figure 1). It was decided, however, that the immunizations would be performed during the follicular phase. This is because it is easier to insert the tool used for flushing the uterus past the cervix follicular phase, and it is necessary to minimize the number of times the animal is anesthetized.

Immunohistochemical localization studies have been carried out to determine the distribution of lymphocytes in the reproductive tract. To date, the 3 cell markers which appear to be recognized by human reagents in baboon are those for B cells (CD3), T cells (CD20) (Figures 2 and 3), and macrophages. Initial measurement of B cell distribution indicates that the number of these cells is different in cervix, vagina, fallopian tube, and uterus. (Figure 4). Quantitative analysis of these studies to determine distribution of immune cells in different reproductive tissues at different stages of the cycle are in progress, but initial examination indicates that the number of B cells in a given tissue is also dependent on the phase of the cycle, particularly in the uterus (Compare figures 4,5,and 6; summarized in figure 7). Additionally, B cells are distributed asymmetrically between the reproductive tract epithelium and lamina propria, dependent on the stage of the cycle (figures 8 and 9). A manuscript describing these results is in preparation.

Part 2 Immunization of baboon peyer's patch (PP) with crude sperm immunogen

6 baboons were immunized with sperm antigen by one of 3 procedures.

Group 1	PP immunization and boost
Pan 1254	Immunized with whole sperm ($3x10^6$/ml) and boosted with sperm extract (350 ug/ml)
Pan 1557	Immunized and boosted with saline
Group 2	IP immunization and boosts
Pan 1670	Immunized with whole sperm and boosted with sperm extract
Pan 1759	Immunized and boosted with saline
Group 3	IP primary immunization, and PP boosts.
Pan 1749	Immunized IP with whole sperm and boosted PP with sperm extract
Pan 1384	Immunized IP and boosted PP with saline

Sperm was collected by electroejaculation, liquified, and washed 3 times with Dulbecco's PBS. PP immunizations were performed via laparotomy. Vaginal and uterine flushes were collected under general anesthesia using 1 ml PBS. Washes were spun at 1500xg, aliquoted, and stored at -20. Protein concentrations in the flushes were determined by Lowry assay, and standardized. It was noted that at the time of the boosts, animals which had received antigen had visibly enlarged lymphatic vessels along the jejunum-ileum area. Those immunized with saline appeared normal.

Antigen binding in the ELISA was optimized by applying

different concentrations of whole sperm to the plate and probing with positive control sera. This sera was from a previous study in which antibodies were raised against the sperm antigen LDH-C4, and known to be reactive with whole sperm in ELISA. The results plateau reached approximately 3.3×10^4 sperm/ml (Figure 10). Approximately the same concentration was determined using sera from the 3 experimental animals in the present study (Figure 11). The remaining assays presented used 5×10^6 sperm/ ml.

ELISAs were performed using sera and vaginal and uterine flushes from immunized animals to determine the levels of IgG, IgM, and IgA elicited against sperm antigen. The data demonstrated no consistent elevation of any of the 3 Ig class titers. While the secondary antibodies used in these assays were shown by immunoblotting to be specific for their respective Ig in sera, there would seem to be considerable cross-reactivity with sperm antigens. While this complicates interpretation of the assay, results still indicate that there is no strong immune response present. Other assays undertaken in an attempt to detect an immune response were immunoblotting of sperm antigens and indirect immunofluorescence, both of which also failed to demonstrate the presence of anti-sperm antibodies.

Problems in generating immune responses against whole sperm or crude extracts may be due to several problems. First, the quantity of significant immunogens in the mixture is unknown. Second, it is difficult with crude antigen mixtures to optimize assays such that relevant antigens are presented properly. Third, it is difficult

to maintain consistency between different antigen preparations. In order to circumvent these problems, we decided to switch to a defined sperm antigen, SP-10. This immunogen addresses the problems outlined above. First, the quantity and purity of the immunogen is easily defined. Second, assay conditions can be optimized for a single protein. Third, bacterially produced recombinant proteins can be generated reproducibly.

Part 3 Induction of immune response by peyer's patch (PP) immunization using SP-10 recombinant antigen.

The immunizations using SP-10 immunogen have shown promising results. A dose response study was initiated in which 3 Peyer's patches in each animal were immunized with 5 mg, 1 mg, 0.5 mg, 0.1 mg and 0.05 mg. Immunizations were carried out without adjuvant.

Animal Number	Primary Immunization 12 Aug, 1993	Booster Immunization 1 Nov, 1993
	5 mg into each of 3 PP	5 mg IM
	1 mg into each of 3 PP	1 mg IM
	0.5 mg into each of 3 PP	0.5 mg IM
	0.1 mg into each of 3 PP	0.1 mg IM
	3 mg IP	3 mg IM

5 animals have been PP immunized using the recombinant sperm antigen SP-10, in collaboration with Dr. John Herr. Examination of sera from animals immunized with various doses (15 mg, 3 mg, 1.5 mg, 0.3 mg, and 0.15 mg injected into 3 PP) indicate that

significant titers have been induced in the higher dose animals (Figure 12). Note that these ELISAs were carried out on serum prior to the boost immunization. Samples are being sent to Dr. Herr's laboratory for independent verification of results. Animals have been boosted IM with the amount of SP-10 used to immunize each PP. Post-boost sera from these animals is presently being examined by ELISA.

Ongoing studies

Animals immunized with SP-10 have been boosted and ELISA assays continue on serum and flushes.

Immunocytochemistry results are being quantified. This will yield the number and size of B and T cells in different reproductive tissues in response to immunization.

IgG has been isolated to sufficient purity to allow gel excision of gamma chains. Secretory IgA has also been isolated. While the molecular weights of the resulting secretory component and alpha chain are slightly higher than expected, repeated isolations have yielded the same results. This may be due to several factors: (1) species variation in the molecular weights (2) incomplete reduction of disulfide bonds (alternate reducing agents are being examined) (3) inaccurate measurement of MW due to the presence of NaCl in the DEAE fractions (4) partial aggregation of heavy chain and secretory component with joining chain (we have observed stubborn aggregate formation in frozen samples). The ratios of the 3 bands secretory component, heavy chain, and light chain) seem consistent with isolated sIgA, as does the comparison of non-reduced vs reduced samples. The isolated protein is also recognized by antibodies against mouse heavy chain.

Impact, Relevance and Technology Transfer.

These findings are very useful at IPR, as several projects involve the induction of immune responses against sperm antigens. This project has optimized assays for detection of these responses, and will soon generate reagents (secondary antibodies) which can be used in both immunocontraceptive development in this department, but as diagnostic tools in other departments as well. Additional methods of immunization planned in the immediate future in other studies at IPR include: 1) oral immunization using antigen entrapped in microspheres, 2) induction of immune responses against recombinant proteins by fusion with promiscuous T cell epitopes. The application of these alternatives are a direct result of these studies.

One technician and one student have been trained in immunization, sera collection, immunoblotting, and ELISA. Two veterinary staff have been trained in identification and immunization of Peyer's patches. The institute has acquired animal cages, electrophoresis equipment and a cryostat which will be of use in these and other ongoing studies investigating secretory immune responses to alternate immunization schemes. Antibodies specific for baboon CD markers have been identified which will be used in several reproductive immunology studies. This includes a CONRAD supported LDH-C4 fertility trial in which secretory immune responses are to be monitored.

Project activities/Outputs

This project attained several targets. First, immune responses have been generated against recombinant immunogens through PP immunizations. Second, the distribution of immune cells has been determined, and alternate immunization routes identified from these results (see proposed extension). Third, we have performed the majority of work necessary in isolating baboon Ig necessary for production of specific immunological reagents.

This project did not, however, accomplish all of the proposed goals. There were two major weaknesses which hampered the proposed research. First was the assumption that commercial human reagents would work well in the baboon. Many human CD markers do appear to recognize baboon lymphocytes, but only in FACS. It has taken some time to determine those commercial reagents which recognize baboon CD markers in embedded tissue. Additionally, while antibodies against human Ig do recognize baboon Ig, the low sensitivity and background recognition of sperm antigens makes the detection of low immune responses, which may have been present, difficult. None of the commercial antibodies against secretory component recognize baboon secretory component (Personal observations and personal communications, Dr. B. Dunbar). This makes measurement of secretory immune responses difficult. As discussed above, these problems will be overcome by the generation of our own secondary antibodies.

Second, while whole sperm or sperm extracts generate

antibodies, they are not truly powerful immunogens. Additionally, performing immunoassays to detect multiple antigens complicates the optimization of assay procedures. While a few antigens may be recognized strongly, one is not sure if they may be membrane bound, cytosolic, acrosomal, conformationally dependent, high or low pI, etc. The use of defined immunogens, as in the present study utilizing SP-10, have greatly simplified detection procedures.

Future Work

Several studies will continue and extend the research begun in this investigation.

1. 5 animals have been immunized with SP-10 via the PP in a dose response study to determine the amount of antigen required in this type of immunization. Analysis of sera following booster immunizations is under way.

2. The distribution of lymphocytes within the reproductive tract will continue using newly acquired antibodies.

4. Isolation of IgM will continue. Heavy chains of IgG, IgM, and IgA, as well as secretory component will be used to immunize goats. These reagents will be useful not only in our own department, but also for other departments and institutions.

5. Mr. Peter Gichuhi is presently a PhD student, and will be using microsphere entrapped antigen in an attempt to generate secretory immune responses. Mr Pius Adoyo, also a PhD student, is also attempting to raise antibodies against sperm antigens. The

expertise gained and reagents generated in this study will be employed heavily in their studies.

6. In a separate but related study, recombinant sperm antigen has been conjugated to promiscuous T cell epitopes to determine the effect on immune responses.

PROPOSED EXTENSION FOR CURRENT GRANT

There is $17,000 left unspent on the present grant. We would like to use these finds to conclude the present studies and would request additional resources to initiate the next logical progression of experiments.

OVERALL AIM

The overall objective of this extension is to bring to a logical conclusion those studies initiated above. First, to generate reagents which will be of use in following immune responses in baboons. Second to apply information gained through immunocytochemistry and PP immunizations in experiments examining the effects of adjuvants and routes of administration on secretory immune responses.

Of the various sperm autoantigens that have been described, SP-10 is one of the most thoroughly characterized at both the molecular and antigenic levels. SP-10 is the immunogen of choice in these studies for several reasons. First, SP-10 antigen is present in the acrosome of all human subjects tested. Second, SP-10 is testis specific (Herr et al, 1989), reducing the probability of undesired side-effects. Third, the antigen persists on the sperm head after the acrosome reaction (Herr et al, 1990). Of

primary importance, a monoclonal antibody against SP-10 (MHS-10) inhibits human sperm penetration of hamster eggs (Anderson et al, 1987). Because of these characteristics, SP-10 has been designated as a primary vaccine candidate by the World Health Taskforce on Contraceptive Vaccines (Anderson et al, 1987). Finally, cDNA for both baboon SP-10 and human SP-10 are available, such that immune responses in both the model species and target species can be closely monitored. Thus the overall objective of this proposal is highly relevant to the contraceptive development program of A.I.D.

Investigations to date with SP-10 have involved PP immunization dose response studies, in which immune responses against SP-10 have been generated. It is apparent that different adjuvants and routes of administration will have to be examined in order to generate sufficient and sustained antibody titers which will be practical for application as an immunocontraceptive.

INNOVATIVE ASPECTS

Recent progress in immunology, particularly in the fields of adjuvant development and secretory immunity, make the examination of sperm vaccines feasible. Systematic examinations of induction of mucosal immunity in non-human primates are, however, lacking. We propose to undertake such a study, in which different adjuvants and routes of administration are tested for the ability to generate secretory immune responses. These investigations are a prerequisite for the initiation of meaningful studies on the

feasibility of sperm antigen based immunocontraceptive vaccines in females.

SP-10, like several sperm based vaccines, shows promise in lower mammals. The ability to extrapolate results between species, however, is very limited, as reproductive processes are by definition, species specific. The examination of immunocontraceptives, as well as adjuvants and routes of administration, must by necessity must be carried out in humans or closely related non-human primates. To date, there has been no systematic examination of the effects of routes of administration and adjuvant composition on secretory immune response performed in the baboon. The acquisition of this data is a prerequisite for the investigation of potential sperm immunogens in the generation of an immunocontraceptive response in females. Our investigation would employ one of the most well characterized potential immunocontraceptive sperm antigens, examined in both the baboon and human. The advantage of using this antigen in the examination of secretory immunity is that (1) SP-10 can be produced in bacteria at relatively low cost, and in highly purified form (2) Many of the immunological assays on anti-SP-10 antibodies have already been optimized and (3) the immunogen has shown contraceptive effects in other mammalian species.

TECHNICAL WORK PLAN

Specific Aim I: Generate high quality reagents for baboon Ig assays.

Baboon IgG and sIgA have been isolated. IgM isolation is under way. Gel purified protein (gamma and alpha chain, secretory component) are ready for immunization to produce anti-baboon Ig antisera in goats. Studies to date have demonstrated the low degree of sensitivity and specificity of human and monkey reagents for baboon Ig, particularly sIgA. It is of primary importance to establish reagents such that secretory immune responses can be specifically monitored. Isolated Ig will be used to immunize goats, and the resultant antisera used to assay baboon IgG, IgM, and sIgA against SP-10 antigen in vaginal secretions and serum.

Specific Aim II: Examine adjuvants and immunization regimes for their ability to generate mucosal immune responses against sperm antigens.

While PP immunization with recombinant SP-10 has been shown to elicit an immune response, this method is clearly not viable for widespread application. It does serve, in many ways, to replicate an ideal oral immunization targeted for the GALT system. The next logical step is to utilize the immunogen in an practical immunization route with the appropriate immunogen. The body of data on route of immunization and adjuvant necessary for induction of secretory immune responses in primates is sorely lacking. We

propose to use SP-10 as the test immunogen in a series of immunizations examining the effect of adjuvant and route of administration on secretory immune responses. Adjuvants to be tested are MDP and ISCOMs (immune stimulating complexes). ISCOMs incorporated SP-10 will be obtained in collaboration with Dr. Morein (see Watson et al, 1992, Larsson et al, 1993; Osterhaus et al, 1992; Fekadu et al, 1992)

Adjuvant	Route of primary immunization (3 mg)	Route of booster immunization (1.5 mg)	
MDP	Oral	Oral	
	Oral	IM	
	Cervical	Cervical	
	Cervical	IM	
	IP	IP	
	IP	IM	
ISCOM	Oral	Oral	
	Oral	IM	
	Cervical	Cervical	
	Cervical	IM	
	IP	IP	
	IP	IM	
NONE	Oral	Oral	
	Oral	IM	
	Cervical	Cervical	
	Cervical	IM	
	IP	IP	
	IP	IM	

Because the route of administration and boost will effect the efficacy of each adjuvant, they must be tested in parallel. To decrease the possibility of the effect of cycle on immune response and subsequent assays, primary immunization will be performed in follicular phase, and boosts at the same point in the cycle approximately 1 month later in each animal. Because each regime will be performed in duplicate, this first set of experiments will be animal intensive. The duplicates will be important to demonstrate that the high response shown in a given animal is not an individual response, but due to the route and adjuvant used. The duration of this portion of the study will, however, be short and a great deal of information obtained. It is imperative to generate as much data from this large set of animals as possible. Serum will be collected 2 times per week. Vaginal flushes and biopsies of reproductive tissues will be collected once per month. The following parameters will be examined:

a. IFN-gamma levels in serum will be assayed to determine which arm of the immune system is stimulated by each regime (TH-1 vs TH-2).

b. IgG, IgM, and IgA collected from sera (2 per week), vaginal flushes and saliva (1 per month) against SP-10 will be subjected to ELISA to determine the levels of each Ig class specific for SP-10.

c. Biopsies from oviductal, uterine, cervical, and vaginal tissues will be taken at the times of primary immunization, booster immunization, and termination of study (projected at

2 months post-boost). Immunocytochemistry will be used to determine the distribution of lymphocytes in reproductive tissues.

Dependent on positive results from this study, the next obvious experiment would be to examine the dose response relationship using the 3 best immunization routes and adjuvant determined above.

MATERIALS AND METHODS

Specific Aim I: Generate high quality reagents for baboon Ig assays.

Standard techniques will be used for IgG, IgM and sIgA isolations. Briefly, IgG and IgM will be partially purified by ammonium sulfate fraction and DEAE chromatography (Dunbar, 1987). Ammonium sulfate is slowly added to serum (40% final concentration) and precipitation allowed to occur for 1 hr with stirring. Precipitate is collected by centrifugation at 4000 x g for 30 minutes, and pellet resuspended in ddH_2O. Sample is dialysed extensively against 0.02 M phosphate buffer, pH = 7.4. This will be applied to DEAE column, and IgG and IgM eluted by step gradient of 0.0 M NaCl, 0.1 M, 0.2 M, and 0.5 M. Fractions will be assayed for the presence of IgG and IgM by SDS-PAGE and immunoblotting. sIgA will be isolated from expressed milk by the procedure found in Johnstone and Thorp, (1987). Briefly, clarified colostrum is

produced by mixing expressed milk (10 ml) with isotonic saline (5 ml) and centrifuging at 100,000 x g for 1 hour. The aqueous layer is acidified to pH = 4.0 with HCl, centrifuged at 30,000 x g for 30 min, and the pellet (mostly casein) discarded. The supernatant is neutralized with 2 M Tris base, and centrifuged as before. The supernatant is filtered (0.45 micron) and applied to a G-200 column (bed volume = 10 x sample volume) and void volume peak eluted with 0.1 M phosphate, pH 6.8. Sample is dialysed against 0.01 M phosphate, 0.1 M NaCl, and applied to ion exchange column (1 ml bed volume per 2 mg protein). Dimeric sIgA is eluted with 0.25 M NaCl. Alpha chain and secretory component will be isolated from bands excised from SDS-PAGE under reducing conditions. As no antibodies are available which are specific for baboon secretory component, its identity will be deduced from migratory characteristics in SDS-PAGE under reducing and non-reducing conditions.

Alpha, gamma, mu, and secretory component will be isolated from partially purified samples by SDS-PAGE under reducing conditions. Bands will be excised, crushed and emulsified in Complete Freunds Adjuvant, and used to immunize goats intradermally (Dunbar and Schwoebel 1990). Animals will be boosted with gel excised bands in Incomplete Freunds Adjuvant and 3 and 6 weeks subcutaneously.

Antibody titers against baboon Ig will be monitored by ELISA. Briefly, plates are coated with 10 - 1000 ng (amount to be optimized) of partially purified IgX (A, G, or M) in antigen coating buffer (0.1 M carbonate buffer, pH = 9.6), overnight at 4

C with shaking (all procedures). Wash plates 4 times with assay buffer (20 mM phosphate, 0.05% Tween 20, and block with 2% BSA in antigen coating buffer overnight at 4 C. Plates are washed 4 times in assay buffer, and serial dilutions of antisera are added and incubated overnight. Antibody is washed off, and biotinylated secondary antibody (anti-goat IgG) added for 1 hour. prepared ABC reagent (Vectastain) is added and allowed to incubate 1 hour prior to washing. OPD substrate is added and incubated for 30 minutes prior to reading.

Specificity of goat antisera will be examined by immunoblotting, using serum and milk proteins separated by SDS-PAGE under reducing (IgG, IgM, and sIgA) and non-reducing (sIgA) conditions. Antibodies from the alpha chain and secretory component should react with the same high molecular weight band (MW = 400-kDa) in immunoblots run under non-reducing conditions, and react with bands at M_R = 58-kDa and 75-kDa under reducing conditions. Bleeds showing high titer (and specificity) antibody will be pooled, aliquoted and frozen for use in ELISA assays. These antisera will also be made available to other institutes maintaining baboons, as the lack of high quality reagents has caused difficulties elsewhere.

Specific Aim II: Examine adjuvants and immunization regimes for their ability to generate mucosal immune responses against sperm antigens.

The stage of the cycle will be determined by assessment of the perineal skin. All immunizations will be performed at the follicular phase as determined by this method. Boosts will be performed 28 days later, at the same stage of the cycle. It is thought that immunization at the same stage of the estrous cycle will give more comparable results than simply relying on time interval between immunizations, as individual cycles may vary by a few days. Immunizations will be formulated by the manufacturers specifications, and the final dose brought up to 0.5 ml. The choice of this volume will necessitate immunization of 3 PP in those groups (each PP can retain 0.2 ml of solution). Animals will be immunized under anaesthesia , and preimmune biopsies, sera and flushes collected at that time.

IFN-gamma levels in serum will be assayed to determine which arm of the immune system is stimulated by each regime (TH-1 vs TH-2).

IgG, IgM, and IgA collected from sera (2 per week), vaginal flushes and saliva (each month) against SP-10 will be subjected to ELISA (Dunbar 1987) to determine the levels of each Ig class specific for SP-10. Titers of antibodies belonging to IgG, IgM, and IgA classes will be determined using second antibodies generated in Specific Aim I.

Biopsies from oviductal, uterine, cervical, and vaginal tissues will be taken at the times of primary immunization, booster immunization, and termination of study (2 months post-boost). Immunocytochemistry will be used to determine the distribution of lymphocytes in reproductive tissues. Biopsies from different portions of the reproductive tract will be formalin fixed and imbedded in paraffin. Antibodies against macrophages, B, and T cells have been shown to be reactive against baboon lymphocytes in fixed tissue.

TIME FRAME

MONTHS		
1-6	1.	Continue IgM isolation, and initiate goat immunizations with secretory component, alpha, gamma, and mu heavy chains.
	2.	Characterize resultant goat anti baboon sera for specificity and sensitivity
6-15	2.	Acquire female baboons, quarantine (3 months) and await resumption of cyclicity (3 months max)
	1.	Perform adjuvant and route of administration study.
	2.	Collect sera (2 per week), vaginal flushes (1 per month), and biopsies (1 per month).
15	3.	Most animals terminated. Those showing sustained titers will be maintained to determine duration of high titers.
15-24	1.	ELISA on serum samples
	2.	IFN-gamma assays on serum samples.
	3.	Immunocytochemistry on sections from biopsies to determine distribution of lymphocytes.

BUDGET: SPECIFIC AIMS I AND II

Description	Unit Cost	Number	Total Cost
Baboon acquisition and quarintine	$420	42	$17,640
Baboon housing (resumption of cyclicity, and study, $2.00/day)	$380 (190 days)	42	$15,960
Assay reagents	$18,000	1	$18,000
Adjuvants	$8,000	1	$8,000
SP-10 production	$4,000	1	$4,000
Salary: Dr. Isahakia	$18,000/year	2	$36,000
Salary: Dr. Schwoebel	$12,000/year	2	$24,000
Salary: 1 Technician	$4,000/year	2	$8,000
Salary: 1 graduate student	$4,000/year	2	$8,000
Capital Equipment Freezer Refrigerator Personal Computer Refrigerated Clinical Centrifuge	 $1,200 $600 $1,500 $3,500	 1 1 1 1	 $1,200 $600 $1,500 $3,500
Books/Journal Subscription	$1,000	1	$1,000
Shipping	$2,000	1	$2,000
Travel	$3,000/meeting	2	$6,000
TOTAL			**$155,400**

Anderson D.J., Johnson P.M., Alexander N.J., Jones W.R., Griffin P.D. (1987). J. Reprod. Immunol **10**:213-257.

Dunbar B.S. (1987). Two-dimensional Electrophoresis and Immunological Techniques, Plenum Press, New York.

Dunbar B.S., and Schwoebel E.D. (1990). Preparation of polyclonal antibodies. Methods Enzymol. **182**:663-670.

Fekadu M., Shaddock J.H., Ekstrom J., Osterhaus A., Sanderlin D.W., Sundquist B., Morein B. (1992). An immune stimulating complex (ISCOM subunit rabies vaccine protects dogs and mice against street rabies challenge. Vaccine **10**:192-7.

Herr J.C., Flickinger C.J., Homyk M., Klotz K., John E. (1990). Biochemical and morphological characterization of the intra-acrosomal antigen SP-10 from human sperm. Biol Reprod. **42**:181-93.

Herr J.C., Wright R.M., Flickinger C.J., (1989). Biochemical, ultrastructural and genetic characterization of human acrosomal antigen SP-10. Proceedings of the 4th International Congress of Reproductive Immunology. Amsterdam: Elsevier.

Johnstone A., Thorpe R. (1987). Immunocytochemistry in Practice 2nd ed. Blackwell Scientific Publications, Oxford

Larsson M., Lovgren K., Morein B. (1993). Immunopotentiation of synthetic oligopeptides by chemical conjugation to iscoms. J. Immunol. Methods **162**:257-60.

Osterhaus A., de-Vries P., Morein B., Akerblom L. (1992). Comparison of protection afforded by whole virus ISCOM vs MDP adjuvanted formalin-inactivated SIV vaccines from IV cell-free or cell-associated homologous challenge. AIDS-res. Hum.Retroviruses **8**:1507-10.

Watson D.L., Watson N.A., Fossum C., Lovgren K., Morein B. (1992). Interactions between immune-stimulating complexes (ISCOMs) and peritoneal mononuclear leucocytes. Microbiol-Immunol **36**:199-203.