SCREENING FOR AUTOANTIBODIES IN HUMAN SUBJECTS IMMUNIZED WITH Pr-β-HCG-TT

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ABSTRACT

Sera from 3 human volunteers immunised with Pr-β-HCG-TT vaccine were tested for possible reactivity with other human tissues. They were found to be negative for antinuclear, antimicrosomal antibodies and rheumatoid factor. The sera from these subjects did not give any reaction with human thyroid, pituitary, parathyroid, adrenal, testes and ovaries as examined by immunofluorescence techniques. They were also devoid of reaction with tissue substrates from baboons, mice and rabbits.

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INTRODUCTION

In certain circumstances tolerance to self antigens may be broken with the development of antibodies to host tissues. Autoimmune diseases may be caused by exogenous factors, such as cross-reacting bacterial infections and chemicals reacting with native components, resulting in production of "non-self" entities. The diverse mechanisms by which autoantibodies are produced in the body are not fully known. In some cases, autoantibodies which are organ specific lead to the pathogenesis of the diseases (e.g. thyroiditis). In other cases, harmless antibodies are produced as a result of injury (e.g. smooth muscle antibodies (1)). It is possible that immunization with Pr-β-HCG-TT which is an altered human protein may result in the production of cross-reacting autoantibodies with a potential of damaging other tissues. It is with this view that the sera from human subjects immunized with this vaccine were screened for the presence of tissue reacting antibodies.

MATERIALS AND METHODS

The sera analyzed were from three human subjects immunized with Pr-β-HCG-TT. More than one sample of blood removed at different periods after immunization were examined.

1. Antinuclear antibodies: Sera from immunized volunteers at 1/5 and 1/10 dilutions were layered on cryostat sections of mouse kidney and liver. The sections were incubated at room temperature (22°C) for 30 min in a humidified box. The slides were dipped in a container with 0.85% NaCl solution. They were washed for 30 min by gentle stirring of the saline with a magnetic stirrer. The sections were then removed and covered with 1/16 dilution of fluorescein conjugated rabbit antihuman gamma globulin (Cappel Labs., Downingtown, PA 19335, U.S.A.). After a further incubation of 45 min at room temperature, the slides were washed with saline as described above. The sections were mounted in glycerol:phosphate buffered saline (1:2) and viewed under darkground illumination in a Carl Zeiss Universal Microscope Hbo 200 with the excitation filter KP 500 and barrier filter 55.

2. Antimicrosomal antibodies: These were determined by tanned red cell agglutination method. Reagents were supplied by Fujizoki, Japan. The sera were diluted 1/10 to 1/640 with normal saline. Tanned red cells (1%) were added to the sera in haemagglutination plates and incubated at 22°C for 18 hours. Haemagglutination titers of 1/10 and above were considered positive.

3. Rheumatoid factor: was tested by Latex agglutination method,using the rapid slide kit supplied by Hyland Div. Trevanol Labs., California. The sera were analysed at 1/10 to 1/80 dilutions. Titers of 1/40 and above were considered positive.

In all investigations, known negative and positive sera were included as controls at the time of study.

4. The cross-reactivity of the sera of immunised volunteers with human thyroid, parathyroid, adrenal, testes and ovaries was examined by immuno-fluorescent techniques by Dr. S. Whittingham, The Walter and Eliza Hall Institute of Medical Research, Melbourne (Table II) and to tissue substrates from baboons, mice and rats by Dr. P.H. Lambert, WHO Immunopathology Reference Laboratory, Geneva (Table III).

RESULTS

Table I shows the results of the probes for antinuclear, anti-DNA and antimicrosomal antibodies. These tests were essentially negative for all sera obtained from immunized subjects. Known patients of systemic lupus erythematosus (SLE) and thyroiditis showed positive reactions under parallel conditions.

Rheumatoid factor was tested for by serial dilution of the sera. Two of the sera showed positive latex agglutination at 1/10, but this was considered insignificant. All sera were thus negative for rheumatoid factor.

The sera from the immunized subject N.D., who had the highest anti-HCG titers were also tested for reactivity to human and animal tissues by Dr. Whittingham and Dr. Lambert, and at AIIMS. The results are given in Tables II and III. No reactivity was observed against any of the tissues examined.

DISCUSSION

Since the vaccine employed renders a human protein immunogenic in the species, it was considered necessary to check whether the antibodies so generated cross-react with homologous components and tissue substrates. The antibodies seen in common autoimmune disorders fall broadly into two categories: (a) antibodies that react specifically with some organs such as in Hashimoto's disease, pernicious anemia (2) and encephalomyelitis; and (b) antibodies that react with components in many tissues such as the antinuclear antibodies seen in lupus erythematosus, rheumatoid factor reactivity in rheumatoid arthiritis, etc. (1). Accordingly, the sera from the three human subjects immunized with Pr-β-

TABLE I: SCREENING OF SERA FROM HUMAN SUBJECTS FOR NON-ORGAN SPECIFIC AUTOANTIBODIES

Name of subject	Days after immunisation	Anti-HCG titers (% ^{125}I-HCG bound at 1:10 dilution)	Antibodies		
			Anti-nuclear	Antimicrosomal	Rheumatoid factor
N.D.	132	41.59%	negative	negative	negative
N.D.	160	43.46%	"	"	"*
N.D.	233	40.19%	"	"	"
A.M.	198	18.55%	"	"	"
K.W.	208	35.97%	"	"	"*
K.W.	243	31.43%	"	"	"
Controls:	Clinical status				
D.E.	Systemic lupus erythematosus	-	positive (1/80)	-	negative
K.L.	same as above	-	positive (1/64)	-	"
I.N.	Normal	-	negative	negative	negative
P.T.	Thyroiditis	-	"	positive (1/80)	"
D.K.	Rheumatoid arthritis	-	"	negative	positive (1/60)

* Showed latex agglutination at 1/10 which is considered insignificant.

TABLE II: REACTIVITY OF ANTI-Pr-β-HCG-TT SERA AGAINST HUMAN ORGANS

Sera	Anti-HCG titers % ^{125}I-HCG bound at 1:10 dilution	Human Organs tested	Observations
1. Normal (non-immunized subject)	Nil	Thyroid Parathyroid Adrenal Kidney Testis Ovary Pituitary*	No reaction seen with any of the organs by immunofluorescence
2. N.D.	43.46		

* Tested at AIIMS. The serum from this subject as well as from A.M. and K.W. taken at time points with highest anti-HCG titers did not give specific fluorescence with human fetal pituitary when tested at 1/4 and 1/8 dilutions.

TABLE III: REACTIVITY OF ANTI-Pr-β-HCG-TT SERUM WITH TISSUE SUBSTRATES FROM VARIOUS SPECIES

Sera	Anti-HCG titers % ^{125}I-HCG bound at 1:10 dilution	Tissues from	Tests	Observations
Normal Human	Nil	Mice	a) indirect immunofluorescence	1.No tissue reacting antibodies detected
N.D.	28.76%	Rabbit Baboon	b) Anti-DNA antibodies	2.No anti-DNA antibodies found

HCG-TT vaccine were tested for both types of antibodies. No evidence of antinuclear, antimicrosomal and rheumatoid factor reactivity was found in any of the sera. The tests employed were sensitive and reliable enough to furnish positive indications in known cases of auto-immune disorders with antibodies reacting with these components. The serum from N.D. with highest titers of anti-HCG was also screened for reactivity by immunofluorescence techniques with a number of human organs, thyroid, parathyroid, adrenal, kidney, testis and ovaries. These tests were performed by S.W. in a laboratory well versed in the detection of tissue reacting antibodies. These conclusions are further supported by the complete normalcy of organ function tests reported in another communication in this series. The sera were also devoid of reaction with tissue substrates from baboons, mice and rabbits.

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