Some results on an immunochemical analysis of fluorescein-conjugated antihuman IgM sera

Some fluorescein-conjugated antihuman IgM sera are capable of demonstrating IgM antibodies to toxoplasma present in infected newborns. Other comparable reagents to human IgM do not. This discrepancy was never observed when IgM toxoplasma antibodies from adult patients were studied. Immunochemical analysis demonstrated anti-IgM, reactive to newborn IgM toxoplasma antibody, to contain additional antibody specificity to μ chain in comparison with the nonreactive antiserum. In view of published findings that μ chain antigenic specificities are represented in each 19S IgM molecule and the high frequency of 7S IgM in newborns, it was tentatively concluded that the reactive anti-IgM serum demonstrates 7S IgM toxoplasma antibodies in the serum of newborns, these being of a μ chain antigenic specificity that is not detected by nonreactive antiserum.

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RECENTLY, Remington[1] reported discrepant results obtained with the (indirect) fluorescent antibody technique, using fluorescein-labeled antihuman IgM sera to detect toxoplasma antibodies in the serum of newborns. The discrepancies consisted of the ability of a particular fluorescein-labeled anti-IgM serum to give positive fluorescence with newborn IgM antibodies, whereas many other fluorescein-labeled anti-IgM sera tested did not. These contrasting results were never obtained when IgM toxoplasma antibodies from adult patients were investigated. This paper describes some results obtained through immunochemical analysis of fluorescein-labeled anti-IgM sera used in the studies mentioned above.*

The following qualities of the discrepant anti-IgM sera were investigated and compared: (1) the anti-IgM antibody titers, (2) the over-all specificity of the anti-IgM sera, and (3) the specificity of the anti-IgM antibodies in particular.

RESULTS

1. Double diffusion experiments in agar gel, using adult serum as IgM antigen source, did not indicate that the discrepant anti-

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IgM sera were of different potency. When serial dilutions of toxoplasma IgM antibody from adult patients' serum were assayed in the fluorescent antibody test, the anti-IgM serum capable of detecting newborn toxoplasma IgM antibody (reactive IgM antiserum) did show the same sensitivity as the anti-IgM serum unable to demonstrate newborn toxoplasma antibodies (nonreactive IgM antiserum). These results made it very improbable that antibody potency differences were the reason for the discrepant observations mentioned in the introduction.

2. Most antihuman IgM antisera need absorption to obtain monospecificity for μ chains. Not only antibodies to serum proteins other than IgM, but also antibodies to light chains are to be removed. The presence of the latter antibodies might cause (false) positive reactions with IgG toxoplasma antibodies. In appropriate double diffusion and immunoelectrophoretic experiments, no contaminating antibodies were detected in either fluorescein-labeled anti-IgM sera. In addition, the presence of specific toxoplasma antibodies in the discrepant anti-IgM sera was excluded.[1] These results lead us to conclude that the discrepant fluorescein-labeled anti-IgM sera are essentially monospecific, i.e., directed to μ chain.

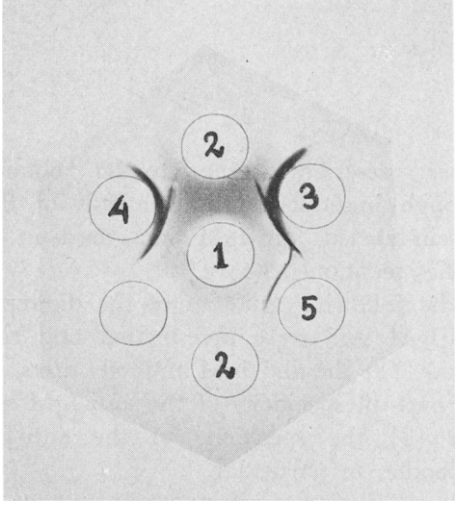

Fig. 1. *Well 1,* reactive antihuman IgM (reacts positively with newborn toxoplasma IgM antibody in fluorescent antibody test); *well 2,* nonreactive antihuman IgM (reacts negatively with newborn toxoplasma IgM antibody in fluorescent antibody test); *wells 3* and *4,* contain pooled normal human serum, diluted 1:8 and 1:16, respectively; *well 5,* antigen JR 36 is "IgM free" pooled cord serum.

3. However, qualitative differences were observed when reactive and nonreactive IgM antisera were analyzed for μ chain specificity. Fig. 1 is representative for the specificity difference observed in many of the double diffusion experiments performed. From Fig. 1 it can be seen that reactive anti-IgM (center well) recognizes more antigen specificity in pooled adult serum than does nonreactive anti-IgM serum. The precipitin pattern can be explained only by the presence of at least two IgM populations, each carrying a different μ chain specificity.

The existence of two (or more) separate μ chain antigenic specificities has been reported earlier.[2, 3] In assuming that reactive anti-IgM reacts with two individual μ chain specificities (no other antibodies than those specific for IgM were found), the reaction of identity (Fig. 1) between the second, additional precipitin band obtained with pooled human serum and the one formed with antigen JR 36 seems controversial. Antigen JR 36 is cord serum from which IgM was supposedly removed. It was prepared for absorption to render (nonreactive) anti-IgM monospecific. Recently, the presence of 7S IgM in cord serum has been reported by Perchalski and associates.[4]

The frequency of 7S IgM in cord sera was found to be relatively high. Antigen JR 36 was made by pooling many different cord sera and, therefore, the presence of 7S IgM in this pool seems to be certain. And, also, it may be concluded that removal of 19S IgM only was inadequate to make cord serum a good absorbing antigen. If the IgM antiserum, before absorption, did contain antibodies to two different μ chains, the absorption with JR 36 must have affected the titers of one or the other, or both, in an unfavorable way. As a result, and demonstrated in Fig. 1, the ability of reactive IgM to recognize (7S) IgM antigen in JR 36 seems no longer a controversial finding.

DISCUSSION

Whether the second precipitin line produced by pooled human serum represents 7S IgM or 19S IgM is not yet clear. Dominguez and associates[5] claimed recently that two antigenically distinct classes of 7S subunits of IgM (based on μ chain specificity) exist on each individual 19S IgM molecule. This conclusion rules out the possibility of obtaining two distinct precipitin lines if one were dealing with 19S IgM antigens only. Although the occurrence of 7S IgM has been reported in a variety of pathologic sera, particularly in those showing increased 19S IgM levels, its frequency and concentration in normal adult sera are supposed to be minimal.[6]

If the conclusions of Dominguez and associates are valid in general, any anti-IgM serum, whether of single or dual μ chain specificity, should be capable of detecting all 19S IgM antibodies. Since the aforementioned discrepancy in the fluorescent antibody test with IgM toxoplasma antibodies from cord serum is a true one, it is tempting to speculate that 7S IgM toxoplasma antibodies of single μ chain antigenic specificity are causatively involved. If toxoplasma antibodies produced in utero are limited to a single 7S IgM subclass, or if production of this antibody in the newborn would persist longer in that particular 7S IgM subclass, the fluorescein-labeled antiserum lacking the homologous antibody would fail to detect such toxoplasma antibody.

Why a similar discrepancy was never observed with toxoplasma antibodies from adult patients is not well understood. It might be that production of 7S IgM antibody in utero is more dominant than in adults, since the response at that early stage of immunological development is mainly or only through the IgM-producing system.

The following experiment is designed to make our conclusions definitely valid. Newborn serum containing toxoplasma antibody will be fractionated by density gradient ultracentrifugation. If the macromolecular fraction shows the different results with reactive and nonreactive anti-IgM sera, we are dealing with 19S IgM toxoplasma antibodies of which each subclass carries its own μ chain specificity. If, in contrast, the 7S fraction would give both the extra precipitin line with reactive anti-IgM serum and would only be positive with the latter antiserum in the fluorescent antibody test, the solution to the problem would be in accordance with all data presently available on human IgM antibody and its antigenic properties.

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DISCUSSION

DR. NAHMIAS. I would like to ask if you had an opportunity to immunoelectrophorese this serum to detect the precise migration of the other component?

DR. PEETOOM. It cannot be recognized upon immunoelectrophoresis; we only get one IgM precipitin band.

DR. NAHMIAS. So presumably the two are migrating with the same gamma M mobility.

DR. PEETOOM. That is correct.

DR. NAHMIAS. Have you had an opportunity, or did Remington, to look for specific antibody to toxoplasma in either the Hyland antisera or in the Behringwerke?

DR. PEETOOM. Yes, the presence of toxoplasma antibodies has been excluded by Dr. Remington.

DR. REMINGTON. First of all, I would like to

thank Dr. Peetoom. This is my first experience with cooperation between academia and industry to produce something like this. I think it is very important. Certainly I cannot afford to do it and I don't think anybody in the audience would be willing to make enough antisera for a large population to use.

I would not like to mislead you by stating that all of the samples of antisera we have tested have not been satisfactory. Dr. Alford sent us an antiserum from another company which proved as satisfactory in every respect to the one we use from Behringwerke. Certain of the Hyland antisera will react with a number of the infants' sera, as I stated before. But what concerns me here is that we will not pick up 100 per cent of the infants with congenital toxoplasmosis at this time using the majority of antisera which are on the market. I receive letters frequently from different university laboratories as well as private laboratories, stating that they are now doing the test routinely. They inform me they are using one or another fluorescein-tagged antiserum which I really feel quite certain will not necessarily be identifying the majority of congenital cases.

DR. NAHMIAS. What does Behringwerke use for absorption?

DR. REMINGTON. I don't know.

DR. NAHMIAS. You are absorbing with cord serum are you not?

DR. PEETOOM. With cord sera free of 19S IgM.

DR. NAHMIAS. The problem I have encountered with herpes is that the Hyland fluorescein-conjugated anti-IgM serum has antibody to herpes. I was wondering if it would be possible to prepare anti-IgM sera which are absorbed with cord serum free of antibodies to a particular organism. Would this be possible?

DR. PEETOOM. Theoretically, yes, however, I believe that if we were required to have all those antigens it would make the reagents much more expensive. I would recommend that individual users ask for samples of different batches that might be available, to see if they are free of a particular antibody specificity which would give false positive reactions. If such lots are not available, the individual investigator might absorb small amounts for his own purpose. You would like to have the reagents ready for use, but I think it is not possible at this moment unless the total amount that would have to be produced would make it economically feasible.

DR. BUCKLEY. I would like to ask: What is the source of your antigen? Is it normal adult serum or is it serum from a patient with paraproteinemia?

DR. PEETOOM. Normal adult IgM. In our case, I believe that the limited specificity of the antiserum is due to 7S IgM left in the absorbing antigen. In other instances, the second specificity may never have been there because of antigen competition. Usually, when we found antibody to IgM and if it reacted strongly, we felt the antisera was adequate. If we had continued immunization, we might have developed the second antibody to IgM as well.

DR. BUCKLEY. If normal gamma M is the immunizing antigen, if immunization is continued for a longer period of time the animal should produce antibodies to both subtypes.

DR. PEETOOM. I am certain the immunizing antigen contains both subtypes, but most frequently the animal determines the type of response.

DR. PAUL HARDY. I have a very simple question. What does the 7S gamma M antibody do when it meets the placenta barrier? Does it go through or does it not?

DR. REMINGTON. It is not known whether the baby produces it or whether it passes from the mother to the child.