REVIEW ARTICLE

Laboratory diagnosis of rubella virus infections

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Rubella, although a mild, inconsequential disease in children, presents a serious medical problem in women of child-bearing age, because of the possibility of severe damage to the fetus by transplacental infection. The congenital rubella syndrome [CRS] has emerged as one of the most important preventable medical conditions in recent years, and the efforts of numerous public health and private practitioners are being devoted to its control by detection and vaccination of rubella-susceptible children as well as adolescents and adults.

The virus diagnostic laboratory plays an invaluable role in the diagnosis, management and prevention of rubella infection, for an accurate diagnosis of this condition cannot be made on clinical grounds alone. While, in principle, the diagnosis of rubella infection by laboratory methods does not differ from that of other viral infections, there are important differences in practice. Although it has been known for some years that rubella virus can be grown in tissue culture, isolation by this technique is of little value from the viewpoint of the practising physician, because results cannot be reported in less than two weeks. The virus diagnostic laboratory, therefore, although willing to attempt isolation of the virus, for example from throat secretions, blood, urine, aborted material or other tissues, prefers to use serological methods of diagnosis. Appropriately chosen serological tests can provide prompt information which will help establish a diagnosis and serve as the basis for appropriate action. In favourable circumstances a helpful report can be issued on a single sample of serum, without the necessity of obtaining the classical "paired" or "two-phase" acute and convalescent sera until recently required as a routine for the serological diagnosis of viral infections.

The function of a virus diagnostic laboratory in the diagnosis, management and prevention of rubella is threefold. First, the laboratory can determine, by the widely accepted hemagglutination-inhibition test, the state of resistance (or "immune status") to rubella of women in the child-bearing age groups by testing a single sample of serum. Secondly, the laboratory can carry out tests to identify current infection with rubella virus, especially in pregnant women thought to have been exposed to infection. In the interpretation of these tests a distinction must be made between an original or primary infection with rubella virus, and a subsequent reinfection, because the clinical consequences of a primary infection, usually associated with viremia, are much more serious than those of a reinfection. Thirdly, the laboratory can provide assistance in determining the response to the attenuated live rubella vaccines being widely used in Canada, the United States and many other countries, by conducting serological tests on selected groups of the population ("serological surveillance").

The purpose of this communication, which is based on many years of experience in the study of rubella infection in the Virus Laboratory of the Ontario Ministry of Health, Toronto, is to assist physicians in interpreting the results of serological tests now available to them not only from public health laboratories but from laboratories in hospitals and from some private laboratories.

Before discussing the choice of tests and their interpretation, a brief review of immunity mechanisms in rubella infection will be presented, as well as a discussion of the basic features of primary infection, reinfection and infection with live attenuated vaccine strains.

Basic features of primary infection, reinfection and vaccination with rubella viruses

The term "primary infection" refers to the events occurring at the time of first infection with "wild" (naturally occurring) rubella virus. Primary infections are usually accompanied by some or all of the well known features of rubella, including lymphadenopathy, viremia, rubelliform rash and the occasional complications of purpura, arthritis and involvement of the central nervous system. Primary infections, as will be detailed below, are associated with development of antibodies of various types, some of which may persist for life and are responsible for resistance to a second clinical attack.

Reinfection is the term applied when a person already showing a measure of immunity, as a result of natural infection or of vaccination, on re-exposure to wild virus again becomes infected. As in other viral infections it is now realized that reinfection is not uncommon in rubella.

In one study performed on a "closed" military population, rein-

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fection was observed to occur in only 3.4% of those naturally immune, compared with 80% of those who had been vaccinated previously.[1] The frequency of reinfection, however, may be much less in a general "open" community.

Information available suggests that reinfection does not occur in those who have a reasonable level of circulating virus-neutralizing antibody in the blood. For convenience, tests for the hemagglutination-inhibiting (HI) antibody are done more frequently than tests for virus-neutralizing (N) antibody, and it appears that reinfection does not occur in those with HI antibody titres greater than 1:64, although one report refers to reinfection in a vaccine with an HI titre of 1:128.[1]

Unfortunately, it is not possible to obtain a true picture of the frequency of reinfection among immune groups because this phenomenon is subclinical and can only be demonstated by isolation of virus from throat secretions or by detection of an increase in antibody titre.

Table I presents the major features that characterize and to some extent differentiate the three conditions in which rubella virus infects man: a primary or original infection, a reinfection in a partly immune person, and infection following use of one of several strains of live attenuated rubella virus as a vaccine.

From Table I it will be seen that the incubation period from exposure to onset of clinical or virological manifestations of infection is considerably shorter in reinfection and following vaccination with attenuated virus, and rash as a rule does not occur.[2] Another important difference concerns viral excretion in the nasopharynx. Viral excretion is short-lived and scanty in amount in reinfection and vaccination, compared with primary infection. The so-called "abbreviated" excretion in reinfection is probably explicable on the basis of the presence of neutralizing antibodies in the IgA fraction of the immunoglobulins in the nasopharyngeal mucous secretions.

Viremia, which is a constant feature in primary infection, has been observed only rarely in cases of reinfection.[2] Transplacental transmission of the virus in reinfection is, therefore, unlikely, the viremia being prevented by pre-existent circulating neutralizing antibody.

In subjects receiving a live rubella vaccine, viremia is a constant feature and can be detected 7 to 13 days after vaccination.[3,4] The frequency of transplacental infection of the fetus with the attenuated vaccine virus has not yet been definitely established, although it has been shown that attenuated virus is capable of infecting the placenta and the fetus.[4-7] The virus has been recovered from cervical secretions of the mother two weeks after vaccination.[8] While its teratogenic potential is unknown, there is evidence that vaccine virus can induce histologic abnormalities in fetal tissue similar to those caused by wild rubella virus.[9] These findings are the basis for the prohibition of giving live virus vaccines to women who may be in the early stages of pregnancy or to married women unless an acceptable contraceptive regimen is strictly followed for at least two months after vaccination.

Further discussion of the differing antibody responses, which concerns mainly the absence of antibodies in the IgM fraction in cases of reinfection, is deferred to a later section of this paper.

Table I
Comparison of salient features in primary rubella infection, reinfection and vaccination with live virus

	Infection with wild virus		Vaccination with attenuated live virus
	Primary infection	Reinfection	
Incubation period	14-21 days	8-10 days*	7-10 days*
Rash	Yes or no	No	No, or rarely yes
Virus excretion from throat	Yes	Scanty	Yes
Duration of virus excretion from throat	1-3 weeks	1-4 days	2-5 days
Viremia	Yes	No	Yes
Transplacental passage	Yes	No	Yes or no
Antibodies in IgG fraction	Yes	Yes	Yes
Antibodies in IgM fraction	Yes	No	Yes

*Assessment of incubation period is based on antibody response, infection being subclinical.

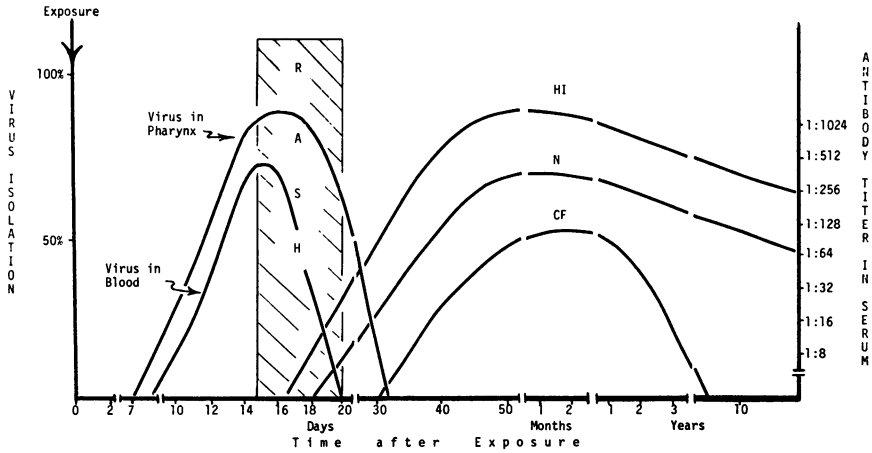

FIG. 1—Virus excretion and antibody response in postnatal primary rubella infections

Antibody response in primary infection with wild rubella virus

During the course of primary infection with wild rubella virus, three major types of antibody develop: neutralizing (N), hemagglutination-inhibiting (HI) and complement-fixing (CF), all of which are associated with both the IgG and the IgM fractions of immunoglobulins. The antibodies associated with the IgG fraction are long-lasting, perhaps for life, while antibodies in the IgM fraction are usually transient. The general behaviour of these antibodies is shown in Fig. 1.

Tests for rubella-specific IgA antibodies, which are the pre-

dominant antibodies of the mucous secretions of the upper respiratory tract, are not at present used in diagnosis, although presence of these antibodies may be important in determining resistance to infection.

Fig. 1 also shows the period of excretion of virus in the pharynx and of viremia.

A. *Antibodies in the IgG fraction of immunoglobulins*

1. *Virus-neutralizing antibody*

Virus-neutralizing (N) antibody, which plays the major role in humoral immunity, is first detected in the IgG fraction about 18 days after exposure during the last stage of the rash, reaching maximum titre about two weeks after the onset of the rash and persisting perhaps for life, with gradual decline in titre (Fig. 1).

2. *Hemagglutination-inhibiting antibody*

Hemagglutination-inhibiting (HI) antibody appears about three days earlier than N antibody and reaches peak at about the same time (Fig. 1). In any one patient the titre of HI antibody is always two- to fourfold higher than the titre of N antibody. Like the N antibody the HI antibody persists for years. The presence of HI antibody, as of N antibody, indicates immunity to rubella virus. Absence of these antibodies is held to indicate susceptibility.

3. *Complement-fixing antibody*

Complement-fixing (CF) antibody in rubella infection appears about four days later than N antibody and about seven days later than HI antibody (Fig. 1). CF antibody reaches its peak titre between one and two months after the onset of the rash; the titre then gradually declines, so that by the end of the third year CF antibody can no longer be detected, or may be present only in a very low titre. In determining rubella immune status, therefore, the absence of CF antibody does not necessarily indicate susceptibility to infection.

B. *Antibodies in the IgM fraction of immunoglobulins*

The same types of antibodies, N, HI and CF, which appear in the IgG fraction are also found in the IgM fraction. The rubella-specific IgM antibodies, unlike those in the IgG fraction, are shortlived, having a half-life of six days.[10] Because of this characteristic, detection of the rubella-specific IgM antibodies plays an important role in the serological diagnosis of primary infection and differentiation between primary infection and reinfection.

Rubella-specific antibodies are detectable in the IgM fraction within two days after onset of the rash, or even earlier.[11] The titre of rubella-specific IgM antibodies remains higher than those in the IgG fraction for about five days after the appearance of the rash. After reaching its peak, 8 to 14 days after onset of the rash, the IgM antibody titre begins gradually to decline and then completely disappears from 20 to 30 days after the onset of the rash (Fig. 2). There is, however, one important exception: a pregnant woman who contracts rubella infection and transmits it to her fetus may have a high IgM antibody level until the time of delivery. This is explained by the persistence of in-

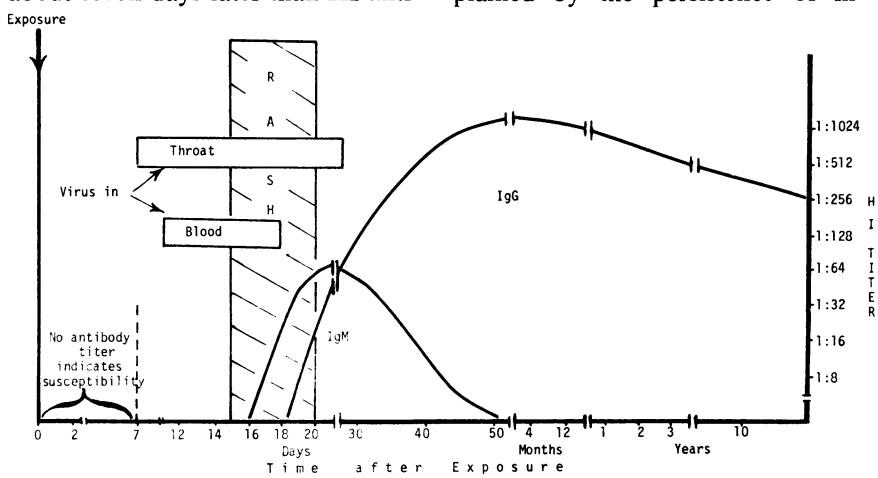

FIG. 2—Rubella HI antibody response in primary infection

fective rubella virus in fetal tissues constantly providing antigenic stimulation to the mother's immune mechanism.[12] Prolonged circulation of rubella-specific IgM antibody has also been reported in cases of thrombocytopenic purpura and carpal-tunnel syndrome complicating rubella.[13]

Because the occurrence of rubella-specific IgM antibodies is transient, their demonstration in a serum specimen, regardless of titre, is indicative of current or very recent infection with rubella virus.

Comparison of antibody responses in IgG and IgM fractions in primary infection, reinfection and vaccination with rubella viruses

Rubella-specific antibodies associated with the IgG fraction of immunoglobulins develop after primary infection, reinfection and vaccination. Rubella-specific IgM antibodies, on the other hand, appear only in primary infection and vaccination but not, as a rule, in cases of reinfection or revaccination.

In the case of reinfection, the "booster" effect results in a much prompter antibody response and the peak titre of the IgG antibodies, which is usually higher than in primary response, is reached much sooner (Fig. 3). Appearance of rubella-specific antibodies in the IgM fraction in cases of reinfection is the exception rather than the rule, and occurs only in individuals whose natural immunity is at the very low level.[14]

In the case of vaccination of susceptible individuals by the usual subcutaneous route, the general pattern of antibody response is similar to that found in cases of primary infection (Fig. 4). The antibodies make their first appearance in the IgG fraction about 7 to 10 days after administration of vaccine and reach their maximum titre two to three weeks later. The rubella-specific IgM antibody response predates that of the IgG by two to three days and the life span of the IgM-associated antibodies is about the same as, or somewhat longer than, in the primary infection, persisting for about eight weeks after administration of vaccine.[15,16]

More recently, a strain of rubella virus vaccine, RA-27/3, developed by Plotkin, has been administered in trials by the intranasal route. Although the pattern of antibody response is the same, the antibodies make their appearance 2 to 3 days earlier than when the vaccine is administered subcutaneously.[16]

Data on antibody response to vaccination of individuals with pre-existing low antibody titres are not available; however, it has been shown in a study by this group that in persons with pre-existing HI antibodies with a titre geater than 1:64, there was no increase in the titre after vaccination.[15]

Antibody response in congenital rubella syndrome

Infants infected *in utero* exhibit a complex pattern of antibodies, both quantitatively and qualitatively. Maternal N, HI and CF antibodies transmitted to the fetus in the IgG fraction normally disappear from the infant's serum within six months of birth.[17,18] In the case of babies infected *in utero,* however, these persist beyond six months at the levels found at birth, or even higher. In addition, cord serum contains rubella antibodies equal to, or greater in titre than, those observed in the mother, as well as unusually high concentrations of IgM and IgA globulins.[17,19]

Congenital infections are also identified by the demonstration in the cord serum or serum from the newborn baby of specific antibodies in the IgM and IgA globulins which are synthesized by the fetus and do not pass the placenta.[17,20] The synthesis of fetal IgM globulins begins some time after the 16th week and possibly prior to the 20th to 24th weeks of gestation.[20] High levels of total IgM in infants may persist into the second year of life,[21] although rubella-specific IgM antibodies may decline within the first year.[22] Rubella-specific IgM antibodies have been demonstrated in 53% of the sera obtained during the first two months of life and in 29% collected in the 3rd to 12th months of life.[23]

In view of these findings, the presence of specific IgM and IgA antibodies in the serum of infants at birth or shortly after provides clear indication of an active immune

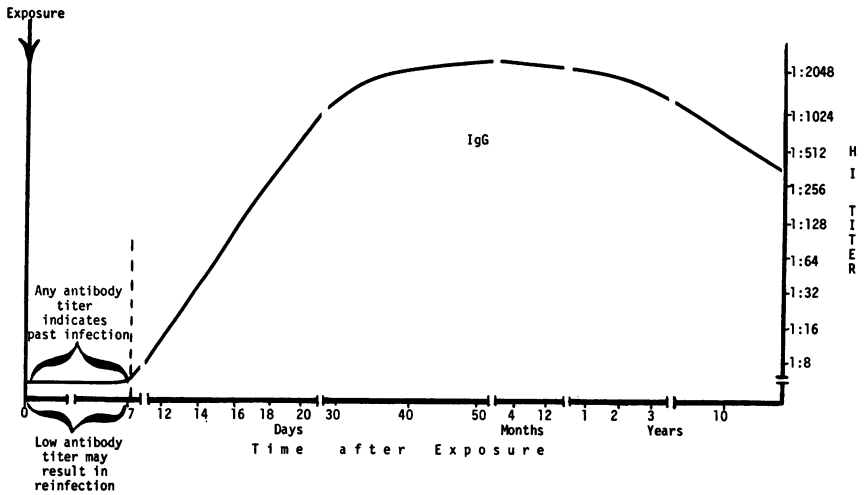

FIG. 3—Rubella HI antibody response in reinfection

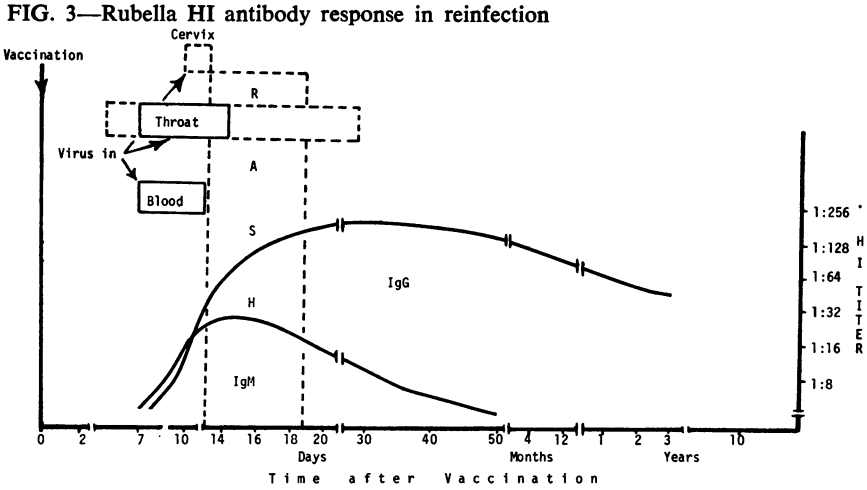

FIG. 4—Rubella antibody response in vaccination

response to rubella infection, against the confusing background of passively transmitted maternal IgG antibodies.

Serological tests for study of rubella infection

There are four serological tests used for the diagnosis of rubella infection: neutralization, complement-fixation, hemagglutination-inhibition and the test for detection of rubella-specific antibodies in the IgM fraction of immunoglobulins. Each test has its own merits and limitations depending on the particular situation.

1. *Neutralization test*

The virus-neutralization test, though specific and reliable in experienced hands, is time-consuming, requiring two weeks to complete. Interpretation of the results of the test is based on the comparison of antibody titres in acute and convalescent serum specimens. A fourfold or greater increase in antibody titre during convalescence is considered diagnostic of infection. Following the isolation of rubella virus[24,25] in 1962 the neutralization test was, for some time, the only procedure capable of furnishing fully reliable information of diagnostic significance. However, with the development of a hemagglutination-inhibition test[26] in 1967 the neutralization test lost its usefulness as a routine diagnostic procedure and is now used only in special cases to confirm results of other tests.

2. *Hemagglutination-inhibition test*

Introduction of the hemagglutination-inhibition test into routine use has, to a great extent, simplified the serological diagnosis of rubella and has made antibody tests possible on a large scale. The test is sensitive and specific, giving highly reproducible results. The results obtained on the same specimen by different laboratories using the same standardized procedure[27] are comparable, indicating the reliability of the test.

As in the neutralization test, the significance of the results obtained with the HI test is based on the demonstration of a significant rise in antibody titre in convalescence.

3. *Complement-fixation test*

Since complement-fixing antibody appears later than the HI antibody, the complement-fixation test finds its best application in cases where two-phase sera are collected at the time when HI antibody level has already reached its plateau (Fig. 1), so that a rise in antibody titre can no longer be demonstrated by that technique.

4. *Test for rubella-specific IgM*

Since the antibody found in the IgM fraction of immunoglobulins is short-lived, the test for the presence of the IgM antibodies is of great value in diagnosing current or recent infection and also for differentiation of primary infection from reinfection. The test has the advantage of giving diagnostically significant results on a single serum specimen.

The test generally used is based on the treatment of test sera with 2-mercaptoethanol (2ME) to denature the IgM fraction and thus reduce the HI titre if the antibody is present in the IgM fraction. Cooper *et al*[28] found that in sera collected within two to three days after onset of the rash a significant drop in the HI antibody titre occurred in all the sera after 2ME treatment. In sera collected four to eight days after onset of the rash the drop in HI titre was observed in 75% of the samples but only in 25% of specimens collected 9 to 15 days after appearance of the rash. From this it is evident that the test is of value for retrospective diagnosis of rubella only during the first week after onset of the rash when 75% of the antibodies are associated with the IgM fraction.

This diagnostic approach has been extensively investigated in our laboratory. The immunofluorescence technique adopted by us for the detection of rubella-specific IgM antibody was found to be very reliable and has been in routine use in this laboratory for the past few years.[11] The test is useful from about one to two days before the rash, prior to the appearance of a detectable level of HI antibody, to about four weeks afterwards. The presence of specific IgM antibody in any titre indicates recent or current primary infection.

Interpretation of results of serological tests

The time of appearance of different

antibodies and their persistence serve as a basis for the serodiagnosis of rubella infection and, because of a number of different situations which arise in the diagnosis of rubella, the application of the various serological tests requires knowledge of their comparative values.

1. *Establishment of immune status*

(a) The absence of HI antibody or N antibody in serum specimens collected within 10 days of exposure indicates susceptibility to rubella.

(b) The presence of antibody at the time of exposure or within 10 days of exposure detectable by any method indicates immunity. This applies only to the cases where the person is intimately exposed to a clinical or suspected case of rubella. In cases of continuous exposure over a longer period, in an environment such as schools or hospitals, when the exact date of exposure is not known, a second serum sample, collected two to three weeks after the first, should be tested. In such cases no change in the antibody titre between the two serum specimens tested simultaneously indicates immunity. If the HI titre, however, is found to be high, then both serum samples should be tested for the presence of CF and specific IgM antibodies. The same CF titre in both serum specimens or absence of CF antibodies and the absence of IgM antibodies confirm immune status.

2. *Establishment of recent infection*

It is important to emphasize that demonstration of N, HI or CF antibodies in a single serum specimen cannot serve as evidence of a recent rubella infection because specific antibodies are persistent.

Various situations encountered in the serodiagnosis of rubella infection are given in Table II. In general, positive serological results are based on the demonstration of the appearance of specific HI antibodies in the convalescent serum sample or demonstration of fourfold or greater rise in titre in the second specimen as compared with the first serum sample. Because of an early appearance and rapid rise of the HI antibody level, in the latter case the interpretation of results may often present difficulties unless the first serum was obtained early

Table II
Significance of rubella antibodies in different situations

Serum obtained	Test: HI	Test: CF	Test: IgM	Interpretation
1.				
1st sample within 10 days of exposure	—			
2nd sample 3 4 weeks later	—			No infection, indicates susceptibility
2.				
1st sample within 10 days of exposure	—			Indicates immunity
3.				
1st sample within 10 days of exposure	−	−		
2nd sample 3-4 weeks later	+	+	+	Primary infection
4.				
1st sample within 10 days of exposure	+	− (or ±)	−	
2nd sample 3 4 weeks later	Fourfold or greater increase	+	−	Reinfection
5.				
1st sample, exposure uncertain	+	+ (or −)	+	
2nd sample 3-4 weeks later	+ (no increase)	Fourfold or greater increase	+(or −)	Primary infection
6.				
1st sample, exposure uncertain	+	+	−	
2nd sample 3-4 weeks later	+ (no increase)	Fourfold or greater increase	−	Reinfection or primary infection since IgM antibodies may already have disappeared
7.				
1st sample. exposure uncertain	+	+	+	
2nd sample 3-4 weeks later	+ (no increase)	+ (no increase)	+	
3rd sample 8 weeks later or longer	+ (no increase)	+ (no increase)	+	Complicated rubella or infected fetus

Table III
Antibodies in congenital rubella syndrome

Serum obtained	Test: HI	Test: CF	Test: IgM	Interpretation
1st sample at birth to fourth months of age	−		−	Rules out congenital rubella
1st sample within first month of life	+	+(or −)	−	
2nd sample six months later	−	−	−	Passively transferred maternal antibodies; rules out congenital rubella
Cord blood	+		+	Intra-uterine infection
1st sample within first month of life	+		+	Congenital rubella

enough after appearance of the rash. This situation, however, may be clarified by employing the CF test. Since the CF antibody response is slower than that of the HI antibody, it may be possible to demonstrate a diagnostically significant rise (fourfold or greater) in CF antibody titre with paired sera which would not show such a rise in the HI test because of the delay in obtaining, the first blood sample (Fig. 1).

Even significant rise of HI or CF antibody titre in the second serum sample, as compared with the first serum specimen, may not always be reliable evidence of recent *primary* infection because the increase of rubella-specific antibodies in the IgG class of immunoglobulins may be due to a "booster" immune response as a result of reinfection. In such cases the test for the presence of rubella antibodies in the IgM fraction of globulins is essential. Since rubella-induced embryopathies are not likely to occur in reinfections, decision to terminate a pregnancy should not be made without first confirming the presence of rubella-specific IgM antibodies. In the case of pregnant women who are suspected of contracting rubella infection during the first trimester, the serum samples in situations 3 to 7, Table II, should always be tested for rubella-specific IgM antibodies to confirm or rule out primary infection since demonstration of rubella-specific IgM antibodies within six to eight weeks of exposure is the only reliable evidence of a recent primary infection with rubella virus.

3. *Congenital rubella syndrome*

Evidence of the synthesis of rubella antibodies by the fetus is the determining factor in serodiagnosis of congenital rubella syndrome.

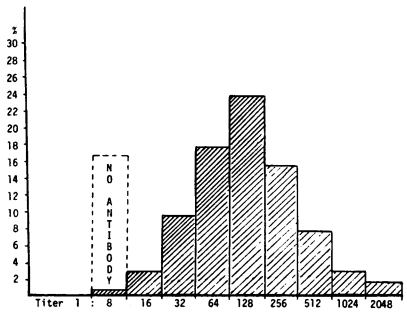

FIG. 5—Distribution of rubella HI antibody titres in 4979 prenatal sera

Serological findings which can be encountered under various conditions and their interpretations are summarized in Table III.

If the serum has HI antibody at birth or during the first month of life and none at six months of age, then the first specimen contained only maternal antibody and the diagnosis of congenital rubella can be excluded.

The absence of antibodies in the serum of infants up to the fourth month of life rules out the possibility of congenital rubella infection. On some rare occasions, however, rubella antibodies cannot be detected in a newborn child exhibiting a rubella syndrome.[29,30] In such cases it is necessary to test a second serum specimen collected at about six months of age.

Demonstration of rubella-specific IgM antibodies in the cord serum provides strong evidence of congenital infection. An unusual amount of IgM and IgA globulins in the cord serum, on the other hand, does not necessarily indicate congenital rubella and may be associated with other congenital infections including syphilis, cytomegalovirus infection and toxoplasmosis.[19,31,32] Presence of rubella-specific IgM antibodies in a mother's blood up to the time of delivery is a strong indication of transplacental infection of the fetus.

Persistence of N, HI and CF antibodies in an infant beyond six months at levels found at birth or higher is suggestive of congenital infection. The strong evidence, however, is provided by the demonstration of rubella-specific IgM antibodies at birth or shortly after. The persistence of specific IgM antibodies is usually limited to the first year of life.

Rubella immune status among adult women in Ontario

A survey conducted in this laboratory during the current year disclosed that 13.3% of women of childbearing age in southern Ontario are susceptible to rubella. The percentage is based on examination of 14,171 prenatal sera. An HI titre of 1:8 or less was used as a baseline of susceptibility.[3,33-37]

The percentage of susceptibles found in southern Ontario closely agrees with that observed in the continental United States and in different countries in Europe where it was found that the percentage of non-immune individuals over 15 years of age varies in a given population between 10 and 20%.[38] However, much higher rates of susceptibility, ranging from 25 to 75%, were reported in certain isolated communities.[39]

To determine the distribution of rubella antibody titres, 4979 of 14,171 prenatal sera tested were titrated for HI antibodies and the results are given in Fig. 5. The percentage of susceptibles in this group was 17.1%, of whom only 0.8% had detectable antibodies at 1:8 dilution. The remaining 82.9% had titres of 1:16 or higher, the majority (57.6%) falling in the range between 1:64 and 1:256. This is in close agreement with the findings in the United States.[36]

Comments

As has been pointed out in this communication, the correct interpretation of the results of serological tests depends entirely on a thorough understanding of immune response in rubella infection. Selection of a proper test, or a combination of tests, is essential in order to establish with certainty primary infection or to differentiate primary infection from reinfection which is of paramount importance in the case of pregnant women exposed to rubella. This can only be done if the laboratory is provided with an adequate case history.

During the past four years primary rubella infection has been diagnosed in this laboratory in 173 women in their first trimester of pregnancy.

In addition to the diagnostic service, the virus laboratory, as of January of this year, commenced testing routinely all prenatal sera to provide physicians with information concerning the rubella immune status of their patients.

With increasing demand for rubella serology and to provide a faster service throughout the province, the Laboratories Branch of the Ontario Ministry of Health installed last January Rubella Diagnostic Units in eight regional laboratories: Hamilton, Kingston, London, North Bay, Ottawa, Thunder Bay, Windsor and Woodstock.

The co-operation of Mrs. Donna J. Reed, Chief, Serology Laboratory, in providing prenatal serum specimens submitted for Rh and syphilis tests, is greatly appreciated.

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They constitute 60% of all ovarian tumours in females under 20, two thirds being dermoids. In premenarchal girls 90% of ovarian tumours are teratomas.[1,6] In adult females this figure drops to between 15 and 20%, with most teratomas in this age group being dermoids.

Teratomas of the ovary may be benign or malignant. The common dermoid cyst is apparently benign, all tissues being well differentiated with no areas of malignant degeneration. Dermoids are cystic tumours with a preponderance of ectodermal elements, although endodermal and mesodermal elements are found. Usually the cysts are filled with a greasy, sebaceous material and large quantities of hair, skin, teeth, bones and neural elements. Many of these growths are asymptomatic and are detected upon routine physical examination.

Malignant teratomas range from the disorganized, anaplastic and highly malignant embryonal teratomas to the partially differentiated and less malignant neoplasms. In the most mature types an imperfect fetus (fetus *in fetu*) may be formed.

Several theories have been proposed to explain the origin of teratomatous tumours.

1. *Incomplete twinning*[3]

This theory postulates that the inclusion of embryonic remnants of one twin in the tissue of another may manifest itself later in teratoid development. In support of this hypothesis an increased frequency of a family history of twins is noted in association with dermoids, and teratomas containing all three germ layers more closely resemble an imperfect attempt to form a human body than do other neoplastic growths. However, this theory cannot explain the predilection of dermoids for the ovary.

2. *Blastomere inclusions*[3]

The claim is made that at an early phase of embryonic development blastomeres still retaining the potential for mutilayered differentiation may be sequestered, to be activated subsequently in the form of teratomas. Again, this theory does not explain the predilection teratomas have for the ovary.

3. *Ovarian pregnancy*[3]

Ovarian pregnancy requires ovarian fertilization and subsequent imperfect development with the formation of a teratoma. A serious objection to this theory, which can also be applied against the theory of incomplete twinning, has been provided by chromosomal studies of teratomatous tumours.[7-10] Examination of 17 such ovarian neoplasms reported in the literature has shown all of them to have a typical female XX sex chromosome constitution. This observation favours development of teratomatous tumours from an endogenous cell line.

4. *Imperfect parthenogenesis*[2]

According to this theory, teratomas are believed to arise from undifferentiated germ cells present in the developing gonadal anlage. During normal development these germ cells originate in the endoderm of the yolk sac and migrate into the gonads at some later time. There, because of unknown inciting factors, they may take part in a process of imperfect parthenogenesis leading to formation of a teratoma. If the theory of imperfect parthenogenesis explains correctly the origin of ovarian teratomas, then it is possible that there may be a genetic defect responsible for inciting parthenogenesis which can be transmitted from one generation to the next.

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Résumé

La fréquence familiale des kystes dermoïdes de l'ovaire

L'article présente le cas d'une mère et de ses deux filles qui présentaient des kystes dermoïdes des deux ovaires et il passe en revue la littérature relative à la fréquence familiale de cette pathologie. Les auteurs décrivent les tératomes, soulignent leur fréquence et rappellent les théories étiologiques.

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