The Immunological Basis for Immunization Series

Module 11: Rubella

Immunization, Vaccines and Biologicals

World Health Organization

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Contents

Abbreviations and acronyms

ACV	acyclovir
BCG	bacille Calmette-Guérin (vaccine)
CMI	cell-mediated immunity
CRS	congenital rubella syndrome
CTL	cytotoxic T lymphocytes
DBS	dried blood spots
DEA	diethylamine
DNA	deoxyribonucleic acid
DR	D-related
EIA	enzyme immunoassay
EPI	Expanded Programme on Immunization
GIVS	Global Immunization Vision and Strategy
GMT	geometric mean titre
HA	haemagglutination
HI	haemagglutination-inhibition
HLA	human leukocyte antigen
IFN	interferon
IgA	immunoglobulin A
IgD	immunoglobulin D
IgG	immunoglobulin G
IgM	immunoglobulin M
IN	intranasal
iu/ml	international units per milliliter
LP	lymphoproliferative
MACRIA	M-antibody capture radioimmunoassay
MHC	major histocompatibility complex
MMR	measles-mumps-rubella
MMRV	measles-mumps-rubella-varicella
MR	measles-rubella

NP	nasopharyngeal
NT	neutralization
PFU	plaque-forming units
PPD	purified protein derivative
PRP	progressive rubella panencephalitis
RNA	ribonucleic acid
RT-PCR	reverse transcription/polymerase chain reaction
RV	rubella virus
SC	subcutaneous
SI	stimulation index
SRH	single radial haemolysis
SSPE	subacute sclerosing panencephalitis
Th	T-helper
TOP	termination of pregnancy
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
WHO	World Health Organization

Preface

This module is part of the series The Immunological Basis for Immunization, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1]. In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme - diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005-2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules - which are published as separate disease/vaccine-specific modules - is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the Vaccine Position Papers (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial Immunological Basis for Immunization series, as well as those involved in its updating, and the development of new modules.

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

1. The disease and virus

1.1 Rubella

Rubella disease is a mild self-limited rash illness that usually occurs during childhood. Rubella virus (RV) is transmitted through person-to-person contact or droplets shed from the respiratory secretions of infected persons. The average incubation period is 14 days with a range of 12-21 days. During the first week after exposure, there are no symptoms. During the second week after exposure, there may be a prodromal illness consisting of low-grade fever (<39.0°C), malaise, mild coryza, and mild conjunctivitis, which is more common in adults. Postauricular, occipital and posterior cervical lymphadenopathy is characteristic and typically precedes the rash by 5-10 days. Children usually develop few or no constitutional symptoms. Rarely, rubella may mimic measles in its severity of fever and constitutional symptoms, but Koplik's spots are absent (Plotkin & Reef, 2004).

At the end of the incubation period, a maculopapular erythematous rash appears on the face and neck. The rubella rash occurs in 50%-80% of rubella-infected persons and is sometimes misclassified as measles or scarlet fever. The maculopapular erythematous rash of rubella starts on the face and neck and progresses down the body. The rash, which may be pruritic, usually lasts between one and three days. It is fainter than measles rash and doesn't coalesce, and it may be difficult to detect, particularly on pigmented skin.

After exposure, virus replication occurs, initially in the nasopharynx. Persons with rubella are most infectious just prior to and soon after the rash has erupted, but virus excretion from the pharynx can be detected from seven days before, to 7-12 days after onset of rash. Viraemia can be detected during the week before onset of rash and disappears with the appearance of rubella-specific IgM antibodies, which appear soon after the onset of the rash (Figure 1).

Rubella disease is usually mild, resulting in very few complications apart from the serious consequences of congenital rubella infection. Transient joint symptoms (e.g. arthritis, arthralgias) may occur in up to 70% of adult women with rubella. They usually begin within one week after rash onset and typically last for 3-10 days, although occasionally they may last for up to one month. Other complications include thrombocytopenic purpura (1 in 3 000 rubella cases) and encephalitis (1 in 6 000 rubella cases). In the recent outbreaks in the Kingdom of Tonga (2002) and the Independent State of Samoa (2003), encephalitis was seen more commonly, with an estimated rate of 1 in 300 to 1 in 1 500 cases (Alan Ruben, personal communication 2007). Long-term sequelae with such progressive rubella panencephalitis (PRP) are rare. PRP has similarities to subacute sclerosing panencephalitis (SSPE) caused by measles.

1.2 Congenital rubella syndrome (CRS)

The most important and serious consequence of rubella is congenital rubella infection. When primary rubella infection occurs in a pregnant woman, the virus can infect the placenta and fetus during the period of viraemia. The risk of congenital infection is related to the gestational age at the time of maternal infection. The outcome of a primary rubella infection during pregnancy includes: spontaneous abortion; stillbirth/fetal death; infant born with CRS; infant born with congenital rubella infection without congenital defects and birth of a normal infant.

The most common defects of CRS are hearing impairment (unilateral or bilateral sensorineural), eye defects (e.g. cataracts, congenital glaucoma, or pigmentary retinopathy), and cardiac defects (e.g. patent ductus arteriosus, or peripheral pulmonic stenosis). Other clinical manifestations may include microcephaly, developmental delay, purpura, meningoencephalitis, hepatosplenomegaly, low birth weight and radiolucent bone disease (Table 1). Children with CRS may develop late-onset manifestations including endocrine abnormalities (e.g. diabetes mellitus, thyroid dysfunction), visual abnormalities (e.g. glaucoma, keratic precipitates), and neurological (e.g. progressive panencephalitis), in addition to developmental manifestations which include autism (Cooper & Alford, 2006).

Table 1: A comparison of prospective studies and data previously reported for selected defects among infants with CRS (adapted from Reef et al. 2000, with permission from the University of Chicago Press)

Clinical manifestations	Number of studies	Subjects with manifestation/total subjects (%)	Percentage from previously reported source*
Hearing impairment	10	68/113 (60%)	80%-90%
Heart Defect	9	46/100 (46%)	-
Patent Ductus Arteriosus	3	9/45 (20%)	30%
Peripheral Pulmonic Stenosis	3	6/49 (12%)	25%
Microcephaly	3	13/49 (27%)	rare
Cataracts	3	16/65 (25%)	35%
Low Birth Weight (<2500 gms)	2	5/22 (23%)	50%-85%
Hepatosplenomegaly	6	13/67 (19%)	10%-20%
Purpura	5	11/65 (17%)	5%-10%
Mental Retardation	2	2/15 (13%)	10%-20%
Meningoencephalitis	3	5/49 (10%)	10%-20%
Radiolucent bone	3	5/49 (10%)	10%-20%
Retinopathy	3	2/44 (5%)	35%

* Cherry JD. Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Infection [table]. In: Feigin RD, Cherry JD, eds. Textbook of Pediatric Infectious Diseases, 3rd ed. Philadelphia, WB Saunders, 1992:1804-1805.

When pregnant women are infected with rubella during the first 11 weeks of gestation, up to 90% of liveborn infants will have CRS; thereafter the rate of CRS declines until 17-18 weeks gestation when deafness is the rare and only consequence. Reinfection with rubella may occur, but if this occurs early in pregnancy, transmission to the fetus is rare, and the risk of congenital rubella defects probably less than 5% (see section 3.3.3).

Infants with CRS may shed RV from body secretions for up to 27 months, although most infants no longer shed after one year of age (Best & Enders, 2007; Grangeot-Keros, personal communication 2007). Infants that shed RV are infectious, and rubella outbreaks have occurred among health-care workers caring for infants with CRS. It is necessary to ensure that persons in contact with these infants (e.g. health-care workers, family members) are immune to rubella either through vaccination or natural infection (Zimmerman et al. 2001).

1.3 Rubella virus

RV is a member of the Togaviridae family and the only member of the genus Rubivirus (Best et al. 2005). The virus is a single stranded ribonucleic acid (RNA) virus measuring 50-70 nm in diameter. There are three structural proteins, E1, E2 and C. The nucleocapsid C protein is found internally. The core is surrounded by a single layer lipoprotein envelope with spiky projections containing the two glycoproteins, E1 and E2.

1.4 Rubella vaccines

Even though the association between rubella infection in pregnancy and congenital defects was documented in 1941, RV was not isolated in cell culture until 1962, and then by two different groups in Washington DC and Boston in the United States of America (Parkman et al. 1962; Weller & Neva, 1962). In the same year a rubella pandemic started in Europe, and spread to the United States in 1963-1964. In the USA it had devastating consequences as an estimated 12.5 million cases of rubella occurred, resulting in 2000 cases of encephalitis, 11 250 abortions, 2100 neonatal deaths and 20 000 infants born with CRS. This pandemic spurred the development of rubella vaccines, and emphasized the need to develop and implement strategies for using the vaccines to prevent this devastating health burden (Cooper, 1975).

1.4.1 Live attenuated vaccines

Several groups were interested in developing a live-attenuated vaccine in the 1960's. Parkman and colleagues were the first to successfully attenuate RV with 77 passages in African green monkey kidney-cell cultures to give the attenuated strain HPV77 (Parkman et al. 1966). Between 1969 and 1970, three vaccines were licensed in the USA, including: HPV-77.DK12 (dog kidney), HPV-77.DE5 (duck embryo) and Cendehill (rabbit kidney) (Preblud et al. 1980). The Cendehill vaccine was licensed in Britain in 1969, and shortly thereafter the RA27/3 vaccine (human diploid cells) was licensed in Europe. In Japan, the initial vaccines licensed were the Takahashi (rabbit kidney) and Matsuura (Japanese quail-embryo fibroblasts) vaccines. Three additional vaccines were licensed in Japan: Matsuba (rabbit kidney); DCRB 19 (rabbit kidney) and TO-336 (rabbit kidney) (Perkins, 1985).

By 1979 all three of the vaccines licensed in the USA were replaced by RA27/3. RA27/3 vaccine generally induces higher antibody titres and produces an immune response more closely paralleling natural infection than the other vaccines. HPV-77.DK12 was withdrawn due to the higher incidence of side-effects as compared to other vaccines.

After the development and licensure of the initial rubella vaccines globally, additional vaccines were licensed in various geographic locations. In 1980, a rubella vaccine (BRD-2) was developed in the People's Republic of China using a local RV strain from a child, isolated in human diploid cells. In a trial comparing the BRD-2 vaccine and RA 27/3 vaccine, the seroconversion rate and mild side-effects were similar (Yaru et al. 1985). In Japan, currently, five different rubella vaccines are in use, including the TO-366 vaccine (Kakizawa et al. 2001). Even though additional vaccines have been licensed and developed, RA27/3 continues to be the most widely used vaccine strain globally.

Rubella-containing vaccine is available either as a single antigen or combined with measles (MR), measles-mumps (MMR), and measles-mumps-varicella (MMRV). Licensed rubella vaccines are administered subcutaneously with at least 1000 plaque-forming units (PFU) at the time of delivery. Rubella and MMR vaccines are freeze-dried (lyophilized) preparations. The vaccines should be stored at 2ºC-8ºC and protected from light. When stored at 4ºC, their potency is maintained for at least five years. For single antigen rubella, MR and MMR, the RA 27/3 vaccine is very stable at -70ºC. The exception to these storage conditions is the MMRV vaccine - varicella-containing vaccine must be kept frozen at an average temperature of -15ºC (+5ºF) or below. The diluent should be kept separately in the refrigerator or at room temperature. MMRV vaccine must be administered within 30 minutes of reconstitution.

Rubella vaccine is usually administered ≥12 months of age, since maternal antibodies have usually disappeared by that age. The seroconversion rate for children ≥12 months is ≥95%. The age at first vaccination does not appear to be as critical for rubella as for measles vaccine. Passively transmitted maternal antibodies to rubella have been found in approximately 5% of infants from 9 to 12 months, and 2% from 12 to 15 months of age. Studies of rubella-containing vaccine administered at 9 to 12 months of age has demonstrated a seroconversion rate of ≥90%. Rubella vaccine is usually offered to children with measles vaccine (MR) or measles and mumps vaccines (MMR).

1.4.2 Inactivated vaccines

Shortly after the isolation of RV, investigators attempted to develop an inactivated virus vaccine, but their attempts were unsuccessful - either the vaccines were not antigenic or if antibodies were produced, it was questionable if the preparation was contaminated with live virus (Sever, 1963). Because of the considerable promise of live-attenuated vaccines, no further attempt was made until the 1980s-1990s. At that time researchers understood the molecular and antigenic characteristics of the RV which might serve as a basis for the development of alternative vaccines (Waxham & Wolinsky, 1985). Experimental recombinant protein and deoxyribonucleic acid (DNA) vaccines have been developed (Perrenoud et al. 2004; Pougatcheva et al. 1999), but it remains to be seen if these induce persistent immunity, or if there is sufficient demand for a non-live vaccine for these to be produced commercially.

2. The immunological response to natural infection

2.1 Antibody responses

2.1.1 Development of methods for detection of rubella antibodies

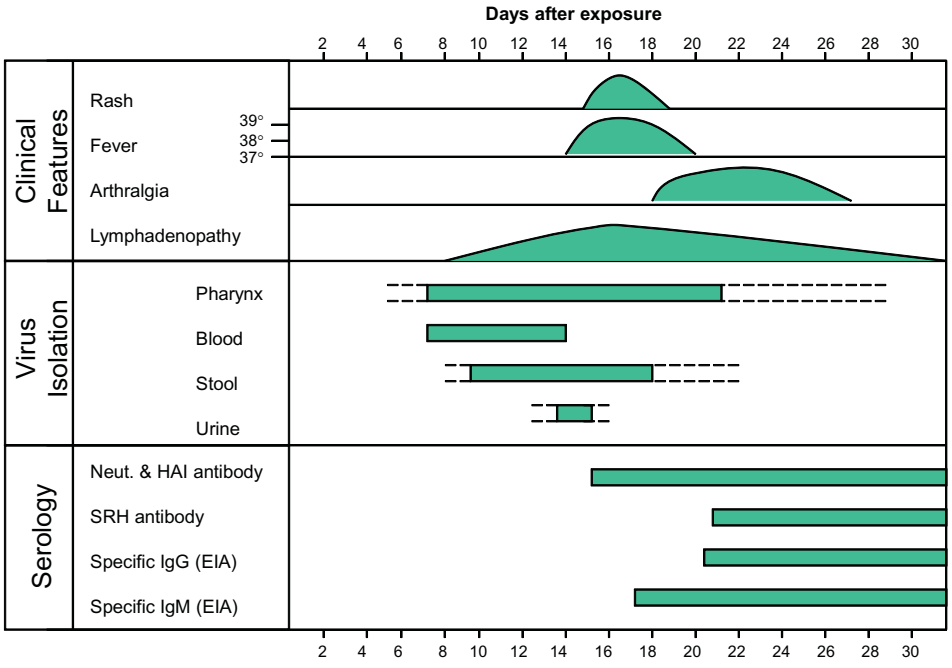
It is necessary to review the development of various methods to detect and quantitate rubella antibodies in order to understand the limitations of early studies in this field. Neutralization (NT) assays were the first to be developed (Parkman et al. 1964), but they are seldom used today as they are demanding and require use of cell cultures. Haemagglutination-inhibition (HI) assays were developed in 1967 (Stewart et al. 1967) and results were shown to correlate well with NT. However, HI is labour-intensive, as serum lipoproteins which inhibit haemagglutination (HA) must be removed from test sera, and antigen used in the test (Stewart et al. 1967). HI is no longer recommended for screening purposes due to occasional false-positive results (PHLS, 1988). More recently, enzyme immunoassay (EIA), single radial haemolysis (SRH) and latex agglutination have been used extensively for rubella antibody screening (Best & O'Shea, 1995; Best & Enders, 2007). Nowadays EIA is the most frequently used test for rubella antibody screening and diagnosis, as it is a sensitive and adaptable technique which can be readily automated. A peptide-EIA has been developed, which employs a synthetic peptide SP15 representing a neutralizing epitope of RV. Results with this EIA compare well with HI (Cordoba et al. 2000).

EIA can also be adapted to detect class-specific antibodies (see 2.1.2) and is the method of choice for detection of rubella-specific IgM (Best & O'Shea, 1995; Best & Enders, 2007). Indirect and M-antibody capture EIAs are available commercially for detection of rubella IgM (World Health Organization, 2007).

2.1.2 Serological responses

HI and NT antibodies develop very rapidly and may be detectable while the rash is still present; specific IgG antibodies detected by EIA and SRH are detectable a few days later (Figure 1).

Figure 1: Relation between clinical and virological features of postnatally acquired rubella



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When class-specific antibodies were examined using EIA or radioimmunoassay, it was seen that rubella-specific IgM appears first and is closely followed by IgG1, IgG3 and IgA (O'Shea et al. 1985; Sarnesto et al. 1985: Wilson et al. 2006). The IgM is transient, it peaks on about day seven, and persists for 4-12 weeks after illness and occasionally for about a year (Al-Nakib et al. 1975; Pattison et al. 1975) (see also section 2.2.1). The predominant response is IgG1, which increases in concentration and avidity within a few days after onset of illness (Wilson et al. 2006). Low levels of IgG4 are occasionally detected, but their detection depends on the antigen used in the assay (Linde, 1985; Thomas & Morgan-Capner, 1988). IgA antibodies persist for at least five years (O'Shea et al. 1985). Rubella-specific IgD and IgE antibodies also increase rapidly and persist for at least six months (Salonen et al. 1985). IgG1 antibodies persist for at least 20 years, and probably for life (Forrest et al. 1971; O'Shea et al. 1985; Toyoda et al. 1999), although they may decline to very low levels in old age. Reinfection (section 3.3.3) is rare in persons with naturally-acquired immunity (Enders-Ruckle, 1969: Enders-Ruckle & Lindemann, 1969).

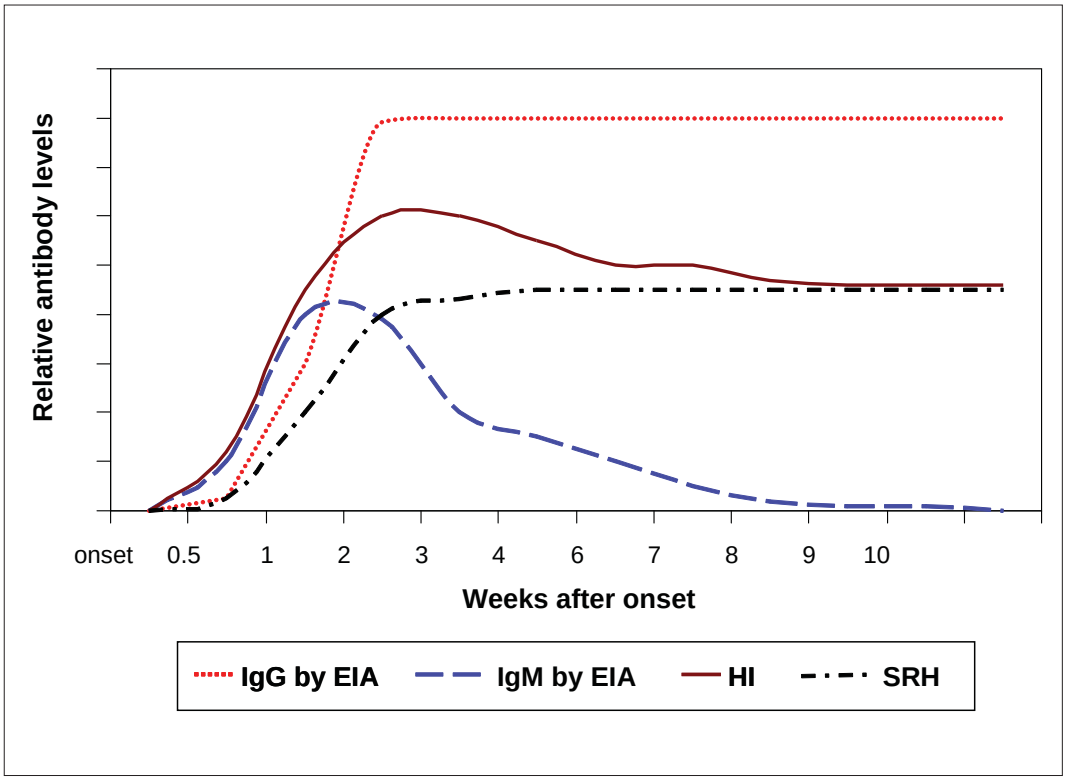
2.2 Laboratory Diagnosis

2.2.1 Diagnosis of postnatally-acquired rubella and assessment of pregnant women in contact with or developing a rubella-like illness

Reliable methods for the laboratory diagnosis of postnatally-acquired rubella are essential because a clinical diagnosis is unreliable, and accurate diagnoses in pregnant women are critical due to the risk of congenital infection in the first 16 weeks of pregnancy (section 1.2). In countries where services are offered for a termination of pregnancy (TOP) when there is a risk of congenital defect, it is necessary to make a diagnosis as rapidly as possible to enable an early TOP. Incorrect diagnosis of rubella can lead to the unnecessary termination of a healthy pregnancy (Best et al. 2002), while a false-negative result can lead to the birth of an infant with congenital defects.

Diagnosis of rubella can be made by detection of rubella IgM, and the detection of a significant rise in rubella-specific IgG concentration (Figures 1 & 2) and by detection of RV in nasopharyngeal secretions by reverse transcription/polymerase chain reaction (RT-PCR) or virus isolation (Best & Enders, 2007; World Health Organization, 2007). To detect a significant rise in IgG concentration, the first serum should be obtained as soon as possible after onset of illness and the second ≥5 days later. Rubella-specific IgM can usually be detected 4-30 days after onset of illness, and often for longer. In a pregnant woman it is essential that a single positive IgM result is confirmed by testing a second serum for rubella IgM and demonstration of a significant rise in IgG concentration where possible (Morgan-Capner & Crowcroft, 2002; Best, 2007; Best & Enders, 2007). It should be remembered that the duration of the rubella IgM response depends on the sensitivity of the assay employed (Tipples et al. 2004; Best & Enders, 2007). Serological testing is essential to confirm or refute rubella infection, as other infections (measles, parvovirus B19, enterovirus, adenovirus, human herpesvirus 6, dengue and group A & C streptococci) may also present with rash and fever (Banatvala & Brown, 2004; da Silva Carneiro et al. 2007; World Health Organization, 2007). It should also be remembered that rubella IgM may be detected after rubella, MR or MMR vaccination (World Health Organization, 2007).

Figure 2. Rubella-specific antibody responses in primary rubella infection



Particular care should be taken when rubella IgM is detected in a pregnant woman with no history of illness or contact with a rubella-like illness, in order to avoid an unnecessary TOP (Best et al. 2002). False-positive results may occur due to the presence of rheumatoid factors or cross-reacting IgM, and such sera may be positive for IgM to a number of different viruses. In addition, long-persisting IgM antibodies may be detected in about 2%-3% of pregnant women for months or years after natural infection or vaccination (Enders, 2005; Thomas et al. 1992; Best & Enders, 2007). In countries where rubella continues to circulate rubella IgM may also be detected early after rubella reinfection, particularly in vaccinees (see section 3.3.3). Tests of rubella-specific IgG1 avidity are usually required in such cases to determine if the IgM is the result of recent primary infection (Hofmann & Liebert, 2005; Best & Enders, 2007; Vauloup-Fellous & Grangeot-Keros, 2007; World Health Organization, 2007). Immunoblotting to detect antibodies to the different structural proteins is also useful (see below), but not widely available.

A number of industrialized countries have produced guidelines for the management of rash illness in pregnancy (Zimmerman et al. 2001; Morgan-Capner & Crowcroft, 2002). Pregnant women exposed to a rubella-like illness should be tested for rubella IgG and IgM as soon as possible after exposure. It is normally advised to test women with a history of rubella vaccination, unless rubella IgG antibodies ≥10iu/ml have been detected previously in a serum taken after documented vaccination. Women with rubella IgG and no rubella IgM can be reassured that there is no evidence of recent primary rubella. Seronegative women and women with antibodies <10iu/ml should be retested at 7-10 day intervals for up to four weeks after contact to ensure that infection has not occurred.

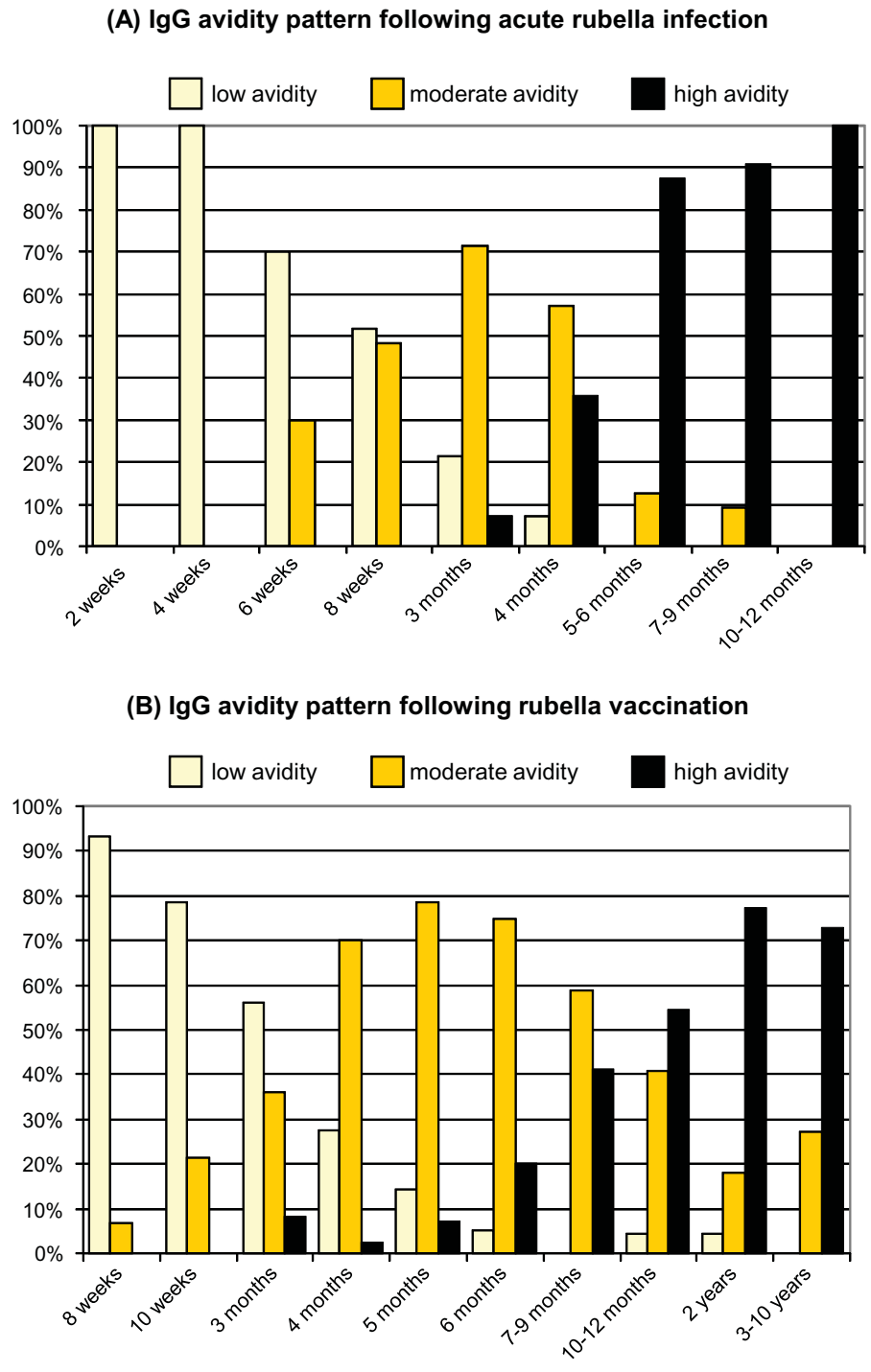
When primary rubella in the first trimester is suspected but cannot be confirmed, and fetal medicine facilities are available, prenatal diagnosis may be considered using amniotic fluid and/or fetal blood (Macé et al. 2004; Best & Enders, 2007). Prenatal diagnosis may also be useful when primary rubella is confirmed between 12-18 weeks gestation, and when rubella reinfection is confirmed before 12 weeks gestation.

2.2.1.1 Avidity of rubella-specific IgG1 antibodies

Assays of IgG1 avidity are useful for diagnostic purposes, particularly to distinguish primary rubella from rubella reinfection and to identify persistent IgM responses and non-specific IgM. The most common diagnostic method employs a denaturating agent (6-8M urea or DEA) to elute low avidity antibody from antigen-antibody complexes in an EIA (reviewed by Thomas et al. 1991; Best & Enders, 2007). Commercial methods are now available and have been evaluated (Eggers et al. 2005; Mubareka et al. 2007).

After primary acute infection, IgG avidity matures from low avidity in the first four weeks, to high avidity in more than 90% of patients four months later when the 35mM diethylamine (DEA) elution method is used (Figure 3). In reinfection, high avidity IgG is already present or will develop more rapidly in the 2-4 weeks after rubella contact (G Enders personal communication, 2006). After rubella vaccination avidity rises more slowly, with high avidity antibody detected in <10 % of vaccinees five months after vaccination, in 20%-40% at 5-9 months, and in 50% at 10-12 months. In approximately 30% of vaccinees, avidity will remain at moderate levels for many years.

Figure 3: Avidity pattern of RV-specific IgG in (A) patients with acute rubella infection (203 sera from 105 patients) and in (B) previously seronegative rubella vaccinees (278 sera from 159 vaccinees mainly vaccinated with RA27/3)



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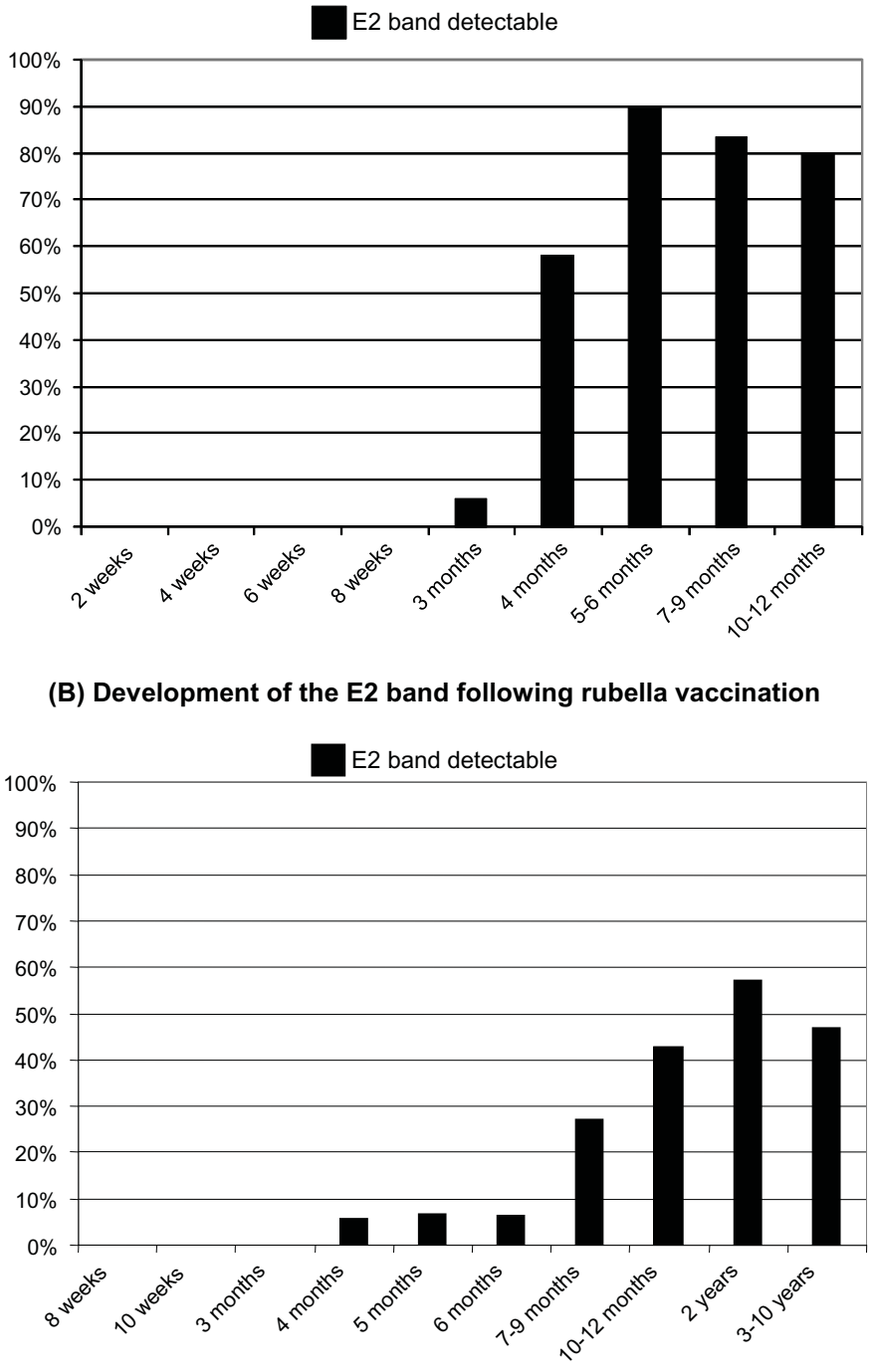
2.2.1.2 Antibody responses to the RV structural proteins E1, E2 and C

Zhang et al. (1992) were the first to report the reliable detection of class-specific antibodies by immunoblot using concentrated whole virus and non-reducing conditions. They demonstrated that specific IgG, IgA and IgM react with E1, E2 and C proteins. IgG and IgA antibodies to E1 appeared between seven and 30 days post onset, but IgG and IgA antibodies to E2 were not detected until one month after onset. Antibodies to C appeared in the acute phase and disappeared by 1-3 years. IgM antibodies to all three proteins were only seen 7-30 days post onset. Weaker reactions are obtained when recombinant proteins rather than concentrated RV are employed for immunoblotting (Nedeljkovic et al. 1999).

The rubella immunoblot has been found to be valuable in the Federal Republic of Germany for distinguishing primary rubella from reinfection. When a commercial rubella immunoblot employing recombinant RV proteins (recomBlot Rubella, MIKROGEN, Germany) is used, an IgG response to E2 is not observed until 3-4 months after onset of illness (Meitsch et al. 1997), and 90% of patients had E2 antibodies by 5-6 months (Figure 4) (Best & Enders, 2007). The slow appearance of E2 antibodies makes this immunoblot method useful for distinguishing primary rubella from rubella reinfection, as the E2 band is seen about one month after contact in reinfection (Best & Enders, 2007).

Figure 4: Immunoblot analysis of the IgG response to recombinant E2 protein in (A) patients with acute rubella infection (255 sera from 129 patients) and in (B) previously seronegative rubella vaccinees (269 sera from 168 vaccinees mainly vaccinated with RA27/3)



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2.2.2 *Diagnosis of congenital rubella infection*

Congenital rubella infection, which includes CRS and congenital rubella infection without defects, is most commonly diagnosed by the detection of rubella IgM in serum or oral fluid taken before three months of age (Eckstein et al. 1996; Best et al. 2005; Best & Enders, 2007). After three months of age the level of rubella IgM declines, but it can still be detected in > 30% infants at 6-12 months of age, when a sensitive assay is used. IgM capture assays are more reliable for this purpose than indirect assays with antigen on the solid phase. If specific IgM is not detected, a diagnosis can also be made by detecting the persistence of rubella IgG antibodies between seven and 11 months of age, or by detection of RV in body fluids or lens aspirates by RT-PCR or virus isolation (Bosma et al. 1995; Jin & Thomas, 2007). Specimens for virus detection are best obtained before three months of age, but RV has occasionally been detected in nasopharyngeal secretions, urine and CSF beyond 12 months, and in a lens aspirate obtained at three years of age.

Laboratory diagnosis of rubella and congenital rubella has been reviewed in detail by Best & Enders (2007).

2.2.3 *Rubella antibody screening*

In some countries women are screened (e.g. in general practice, premarital screening or antenatal visits) for rubella antibodies, to identify susceptible women who should be offered vaccination (Zimmerman et al. 2001; UK Department of Health, 2003). It is important that women who have immigrated from countries without vaccination programmes are tested (Tookey et al. 2002; UK Department of Health, 2003; Robert-Koch-Institut, 2004), or vaccinated if there is no contraindication.

Commercially available EIAs are most commonly used for rubella antibody testing, and most large laboratories in industrialized countries use fully automated equipment. Latex agglutination and SRH may also be used for confirmatory purposes. Initially 15 iu/ml was accepted as the minimum immune titre, when HI was replaced by other techniques (e.g. SRH, latex agglutination and EIA) for rubella antibody screening, as this corresponded to an HI titre of 8-16 (Bradstreet et al. 1978; Kurtz et al. 1980). However, further epidemiological analysis has led to adoption of 10iu/ml as the positive/negative cut-off for rubella IgG antibody detection (Skendzel, 1996; UK Department of Health, 2003).

The presence of rubella-specific IgG does not exclude recent infection, and further tests are required if a woman has a history of rash or contact with a rash. Sera sent for screening should not be tested for rubella IgM, unless there is a history of rash or contact with rash, as this will lead to the detection of occasional non-specific IgM and long-persisting IgM results which are difficult to interpret (de Ory et al. 1998; Vauloup-Fellous & Grangeot-Keros, 2007).

Dried blood spots (DBS) have been used successfully for detection of rubella IgG and IgM, although they provide slightly lower sensitivity when compared with serum (Helfand et al. 2001; Karapanagiotidis et al. 2005). They can be stored at room temperature, and packing and transportation is easier than for serum specimens (Parker & Cubitt, 1999; World Health Organization, 2007).

2.3 Mucosal antibodies and antibodies in other body fluids

Specimens other than serum have been evaluated for epidemiological purposes and diagnosis. Oral fluid and urine, which do not require invasive techniques and sterile syringes for their collection, are particularly useful for epidemiological studies in children and in developing countries.

2.3.1 Oral fluid

Oral fluid, which consists of crevicular fluid and secretions from the salivary glands, can be collected using commercial absorbent devices (Vyse et al. 2001). Rubella-specific IgG and IgM antibodies reflect those in serum, although at lower concentrations (Mortimer & Parry, 1991; McKie et al. 2002) they are usually detected by antibody-capture methods (Perry et al. 1993; de Oliveira et al. 2000). In seroepidemiological studies, the IgG assay performed on oral fluid had an overall sensitivity of 79% and specificity of 90% when compared to serum, but sensitivity declined with age, to only 59% in the 40-49 year age group (Nokes et al. 2001). Therefore, the current assay is not sufficiently sensitive and specific for rubella susceptibility screening of women.

Oral fluid collected between one and five weeks after onset of rash can be used to detect rubella IgM for diagnostic purposes (Perry et al. 1993; Vijayalakshmi et al. 2006). It may also be used to detect rubella IgM in congenital rubella (Eckstein et al. 1996) and to measure specific IgG avidity (Akingbade et al. 2003).

2.3.2 Urine

Rubella IgG antibodies in urine detected by a commercial EIA kit showed a sensitivity of 96.2%, a specificity of 99%, and concordance of 97.2% when compared with serum antibodies detected by a different EIA (Terada et al. 2000). Some experts suggest that this assay can be used for screening and epidemiological purposes, but further evaluation would be required before it can be used for diagnosis of infection.

2.3.3 Nasopharyngeal (NP)

O'Shea et al. (1985) measured rubella-specific IgA and IgG in nasal washings and showed that NP IgA could generally be detected six weeks after onset of illness, and in some cases for between one and six years. Rubella-specific NP IgG could be detected in some volunteers for at least two years. These assays have not been used for diagnostic purposes, but the results are of interest to those developing aerosol vaccines (section 3.1.5.2).

2.4 Cell-mediated immune (CMI) responses

Although CMI responses have been studied in some detail for other viruses, such as measles, there are few recent studies on CMI to rubella. This may be because assays for CMI have been much more demanding than serology, and also because funding for rubella research is difficult to obtain at a time when rubella is being eliminated from industrialized countries.

Rubella infection induces a fall in total leukocytes, T-cells and neutrophils, and a transient depression in lymphocyte responses to mitogens and antigens (e.g. purified protein derivative - PPD) (Buimovici-Klein et al. 1976; Maller et al. 1978; Niwa & Kanoh, 1979; Hyypia et al. 1984), and suppression of bacille Calmette-Guérin vaccine (BCG)-induced hypersensitivity (Ueda et al. 1979; Mori & Shiozawa, 1985), but the mechanism responsible for the mild immunosuppression has not been elucidated. Studies of cytokine secretion demonstrate the strongest responses in persons with a recent history of rubella (Honeyman et al. 1974; Buimovici-Klein & Cooper, 1985). A mixed Th1/Th2 response is seen, as serum interferon-γ is elevated during acute rubella (Hari et al. 1999) and an increase in serum interleukin-10 (IL-10) levels has been demonstrated in children during the first four days after onset of illness (Akaboshi et al. 2001).

Lymphoproliferative (LP) assays show that CMI responses develop a few days after onset of rash and persist at low levels for many years (Buimovici-Klein & Cooper, 1985). Lymphoproliferative responses against E1, E2 and C have been detected. The strongest responses are against E1 (Chaye et al. 1992). Toyoda et al. (1999) showed a positive correlation between HI titres and T-cell activation.

The role of CMI in protection from rubella has not been determined. However, at least 17 antigenic domains, which stimulate major histocompatibility complex (MHC) class II-restricted CD4+ helper T-cells, have been identified within the C, E2 and E1 proteins (Lovett et al. 1993; McCarthy et al. 1993; Chantler et al. 2001). A few studies have identified RV-specific MHC class-I-restricted CD8+ cytotoxic T lymphocytes (CTL; Lovett et al. 1993; Ou et al. 1994). It has been suggested that certain human leukocyte antigen (HLA) phenotypes are associated with poor antibody responses to RV (Ishii et al. 1980; Kato et al. 1982), but this requires further study.

3. The immunological response to immunization

3.1 Serological responses

The majority of early studies on rubella vaccines used HI to measure immune responses (Proceedings of the International Conference on Rubella Immunization, 1969). All licensed rubella vaccines induce a good antibody response, although titres are usually ¼ to ⅛ the level of those following natural infection. The RA27/3 strain induces an antibody response that most closely resembles natural infection, which is why this strain is now the most widely used. The Cendehill strain induces lower antibody levels (Best et al. 1974; Chu et al. 1988). HI antibodies usually develop 10-28 days after vaccination (Meegan et al. 1983; Enders, 1985; O'Shea et al. 1985), although they may occasionally be delayed. It is advised to wait until eight weeks after immunization if it is necessary to detect seroconversion. Approximately 5% of vaccinees fail to seroconvert; this may be due to concurrent infection or to a low level of pre-existing antibodies. Serologic testing after vaccination is not standard practice, however if a vaccinee fails to seroconvert, they should be offered a second vaccination. In general, further testing and vaccination of such individuals is not advised because failure to respond to a second dose is probably due to pre-existing immunity. It is worth re-testing adult women who fail to respond with a different and more sensitive EIA.

NT antibodies were detected at eight weeks and two years after immunization, significantly more frequently in RA27/3 vaccinees (95% and 100%) than in Cendehill vaccinees (56% and 82%), although all vaccinees developed HI antibodies (Grillner et al. 1975). NT antibodies were detected 11 years after RA27/3 vaccination in all 35 vaccinees tested by Horstmann et al. (1985), although antibody titres had declined. Some of the HPV77.DE5 vaccinees failed to develop NT antibodies and some had lost detectable NT antibodies within 3-9 years after vaccination. Just et al. (1985) showed that 307 of 319 (96.2%) women tested 15 years after Cendehill vaccination had NT antibodies. In a more recent study, seroconversion was confirmed by plaque neutralization in 91%-100% MMR vaccinees (RA27/3 rubella strain; Tischer & Gericke, 2000).

Currently, rubella vaccine is usually combined with measles, or measles and mumps in MR and MMR vaccines (section 1.4). Rubella immune responses are not affected by the other components (Weibel et al. 1980; Miller et al. 1995; Crovari et al. 2000). MMR and varicella vaccines have also been successfully combined as MMRV (Nolan et al. 2002; Lieberman et al. 2006). There is no contraindication to giving MMR or MMRV to persons with immunity to one or more of their components (Plotkin & Reef, 2004).

3.1.1 Class-specific antibody responses

Rubella-specific serum IgG, IgA and IgM, and NP IgG and IgA have been detected after vaccination with RA27/3, Cendehill, HPV77.DE5 and TO-336 vaccine strains (O'Shea et al. 1985). Rubella IgG antibodies persist indefinitely with a gradual decline in antibody concentration in most vaccinees (section 3.1.4); specific IgG1 and IgG3 antibodies have been detected (Lehtinen, 1987). Rubella IgM can be detected between three and eight weeks after immunization when sensitive techniques are used (Mortimer et al. 1984; Meegan et al. 1983; O'Shea et al. 1985). Low levels of specific IgM have been detected for up to three years after immunization using M-antibody capture radioimmunoassay (MACRIA) (Best, 1991). Rubella serum IgA may persist for at least 7-9 years after immunization (O'Shea et al. 1985). NP IgA can be detected in nasal washings from most vaccinees at six weeks after immunization, and in some RA27/3 vaccinees for 4-6 years. Lower concentrations of NP IgA were found in Cendehill, HPV77.DE5 and TO-336 vaccinees and were only detected for 2-3 years (O'Shea et al. 1985). NP IgG was detected in 40% of vaccinees at six weeks and in occasional vaccinees at 2-6 years. Rubella IgG and IgA have also been detected in urine after rubella vaccination (Takahashi et al. 1998).

3.1.2 IgG avidity

IgG avidity matures more slowly after vaccination than after natural infection (Hedman et al. 1989; Thomas et al. 1995; Nedeljkovic et al. 2001), but results of rubella-specific IgG avidity tests will depend on the technique used. Enders, using the DEA elution method, detected high avidity rubella IgG in <10% females five months after RA27/3 vaccination and in 50% at 10-12 months (Figure 3) (Best & Enders, 2007). Approximately 30% of vaccinees will not develop high avidity IgG for many years (G. Enders personal communication, 2006), which may make it difficult to interpret avidity results in women with vaccine-induced immunity.

3.1.3 Antibodies to the structural proteins

Antibodies to E1, E2 and C have been detected after RA27/3 immunization (Cusi et al. 1988, 1989; Nedeljkovic et al. 1999), but, as with natural infection, their appearance and persistence depends on the technique used for their detection. Using a commercial immunoblot, <60% vaccinees develop antibodies to E2 in contrast to natural infection where 90% patients will have antibodies to E2 by 5-6 months after infection (Figure 4).

3.1.4 Long-term persistence of antibodies

Studies on the long-term persistence of antibodies after rubella immunization of susceptible persons, have shown that immunity probably persists for life in the majority of vaccinees. Although antibody concentrations fall over time, sometimes to very low levels, immunological memory persists, and a secondary immune response will occur on exposure to rubella (section 3.3.2).

Follow-up studies have shown that 95%-100% RA27/3 vaccinees are seropositive 10-21 years after immunization (Table 2), and there have been similar results with other vaccines (Chu et al. 1988; Best, 1991). Some of these studies were carried out in the USA where there was little circulating RV, while others were conducted in European countries where RV continued to circulate and may have boosted antibody levels (Best 1991). RA27/3 vaccinees tend to have higher antibody concentrations, are less likely to become seronegative, and to have serologic evidence of reinfection than Cendehill and HPV77.DE5 vaccinees (Enders & Nickerl, 1988; O'Shea et al. 1988). Asaki et al. (1997) found that four out of 26 (15.4 %) Matsuba vaccinees living in an isolated community had low levels of antibody (HI <8) 23 years after immunization. About 10% of vaccinees show faster decline of antibodies to low levels. Thus, 6-8 years after vaccination, O'Shea et al. (1982) detected antibodies < 15iu/ml more frequently in Cendehill than in HPV77.DE5 or TO-336 vaccinees, and these low levels were not seen in RA27/3 vaccinees. The positive/negative cut-off used in assays also varies, and so there is a need for EIAs to be standardized (Chu et al. 1988; Best & Enders, 2007).

Table 2: Long-term persistence of rubella antibodies after vaccination with the RA27/3 strain

Reference	Country where study carried out	Years after vaccination	No. seropositive/no. tested (%)	Serological method used
Christenson & Böttiger, 1994	Sweden	16	184/190 (96.8%)*	HI
Enders & Nickerl, 1988	Germany	14	115/115 (100%)	HI
Hillary & Griffith, 1988	Ireland	15	20/21 (95.3%)	HI
Horstmann et al. 1985	USA	11-12	35/35 (100%) 33/35 (95%)	NT HI
O'Shea et al. 1988	UK	10-21	47/48 (97.9%)	SRH, EIA, latex
Zealley & Edmund, 1983	UK	12	93/94 (99%)	SRH
Plotkin & Buser, 1985	USA	12-14	29/29 (100%)	HI

Population-based studies carried out in the USA before rubella was eliminated showed a significant decline in rubella antibody concentrations in children in the 15 years after a single dose of rubella-containing vaccine (King et al. 1993). Orenstein et al. (1986) detected very low concentrations (<7iu/ml) in 8.7% children given HPV77.DE5 10-14 years earlier. In a study by Johnson et al. (1996) 33% of children had rubella antibodies <6iu/ml by EIA, and 37% had no neutralizing antibodies 10-12 years after the first dose of MMR, although at 3-5 years post-vaccination, 90% had EIA antibodies and 100% had NT antibodies. All developed rubella antibodies after a second dose of MMR. It is not clear if some of the seronegative children were primary vaccine failures. In this study, and that by King et al. (1993), it is probable that some children had received HPV77.DE5 rubella vaccine and not RA27/3, which replaced HPV77.DE5 in 1979. Evidence suggests, as discussed above, that antibody responses are more sustained after RA27/3 immunization. It is encouraging that in the USA during 1999-2004, 96.2% of children aged 6-11 years, and 93.7% adolescents aged 12-19 years, had rubella antibodies >10iu/ml (Hyde et al. 2006).

In Newfoundland, Canada, 13.8% of children had antibodies < 10iu/ml 4-16 years after a single dose of MMR containing RA27/3 rubella vaccine (Ratnam et al. 1997). In the Republic of Korea, where rubella continued to circulate, 19% of children had rubella antibodies < 10iu/ml three years after rubella vaccination with Takahashi or Matsuura strains (Ki et al. 2002).

In summary, the very high response rate to a single dose of rubella vaccine (>95% when administered after 12 months of age), an estimated herd immunity threshold for rubella of 83%-85% and the long-term persistence of protection in the majority of vaccines, do not support the need for a second dose of rubella vaccine. However, based on the indications for a second dose of measles- and mumps-containing vaccine, a second dose of MMR is now offered in most industrialized countries, and this helps to boost low rubella antibody concentrations (Johnson et al. 1996; Tischer & Gerike, 2000; Kremer et al. 2006). Davidkin et al. (2008) followed up 90 initially seronegative children who had received two doses of MMR. Twenty years after the first dose all children had rubella antibodies ≥4 iu/ml, but 36% had low concentrations <15iu/ml (Davidkin et al. 2008). Based on the general principle that live virus vaccines not administered at the same time should be separated by at least one month, the minimum recommended interval between the first and second dose of MMR vaccines is one month. The minimum interval between doses of MMRV vaccines is three months (Centers for Disease Control, 2006).

3.1.5 Other routes of administration

3.1.5.1 Intranasal (IN)

RA27/3 is the only vaccine strain that can be administered by the IN route. Studies of the antibody responses induced are of interest to those developing rubella aerosol vaccine. Serum and NP antibodies are similar to those induced by subcutaneous (SC) immunization (O'Shea et al. 1985; Plotkin & Reef, 2004). This method has not been used routinely due to frequent vaccine failure in children, possibly due to the practical difficulties of administering vaccine into the nose (Paradise et al. 1984).

3.1.5.2 Aerosol

Rubella vaccine is not licensed for aerosol administration, but recent trials of aerosol rubella, MR and MMR vaccines have been carried out. The World Health Organization (WHO) has a development project for measles aerosol immunization (Coates et al. 2006), and it would be desirable to administer rubella with measles. Sepúlveda-Amor et al. (2002) compared MR immunization via aerosol and SC routes, and showed similar rates of rubella seroconversion and geometric mean titres (GMTs) in children; reactogenicity of the aerosol vaccine was significantly lower. de Castro et al. (2005) demonstrated more frequent rubella seroconversion and significantly higher GMTs in adults following aerosol MMR than with SC immunization. The development of rubella NP antibodies after aerosol immunization has not been studied, but significantly higher concentrations of measles-specific NP antibody were reported after aerosol than following SC measles immunization (Bellanti et al. 2004).

3.2 Cell-mediated immunity following immunization

The manufacturers of viral vaccines have usually relied on specific antibodies as a surrogate marker of protection and there are few recent studies on CMI following rubella vaccination. Studies carried out in the 1970s and 1980s showed that lymphoproliferative (LP) responses could be difficult to detect, because they are lower than after natural infection (Honeyman et al. 1974; Rossier et al. 1977; Buimovici-Klein & Cooper, 1985). More recently Dhiman et al. (2005) detected rubella-specific LP responses (SI >3) in only 33% MMR vaccinees and stimulation indices (SI) were lower (median SI 2.29) than the responses induced to measles and mumps. These vaccinees were children who had received two doses of MMRII of which the first was 11-17 years previously. Toyoda et al. (1999) measured T-cell activation by expression of CD25+CD45RO+CD4+ T-lymphocytes, which was shown to persist for ≥20 years in vaccinees with HI titres ≥16 after rubella or MMR vaccination in Japan (rubella strain not stated).

A transient immune suppression has been noted following rubella vaccination. It is not as severe as after naturally-acquired rubella and measles vaccination, and unlikely to be of clinical significance. Immune suppression, demonstrated by depressed lymphocyte responses to phytohaemagglutinin, was seen more frequently in RA27/3 vaccinees than following Cendehill or HPV77.DE5 vaccination (McMorrow et al. 1974; Vesikari, 1980; Pukhalsky et al. 2003).

A small number of studies have shown an association of certain HLA alleles with low antibody responses to rubella vaccination (Kato et al. 1982; Ovsyannikova et al. 2005; Ovsyannikova et al. 2006). Ovsyannikova et al. (2007) have also examined the association of HLA polymorphisms with production of IFN-γ and IL-10 as markers of Th1 and Th2 cell activation, and have suggested that HLA genes influence these responses to rubella vaccine.

The association of HLA with the occurrence of joint symptoms in female vaccinees is also of interest. A study in Canada showed that joint symptoms were associated with certain HLA class II alleles (DR2 and DR5) (Mitchell et al. 1998). An earlier study by Harcourt et al. (1979) did not find an association with HLA class I, but did note an association with the menstrual cycle, suggesting that hormonal factors play a role.

3.3 Protection after immunization

3.3.1 Protective efficacy

The protective efficacy of rubella vaccination has been determined in outbreak situations and by experimental challenge studies. The clinical protection afforded by different rubella vaccines in outbreaks was 90%-100% (Table 3). Protection has been noted 2-3 weeks after immunization, as, during outbreaks, cases did not occur in vaccinated individuals after that time (Grayston et al. 1969; Furukawa et al. 1970). Reinfection is discussed in section 3.3.3. Studies in which sub-clinical reinfection following rubella exposure was confirmed serologically are shown in Table 4. In some studies, more than 40% of HPV77 and Cendehill vaccinees experienced sub-clinical reinfection, but it has been observed in only 7%-10% of RA27/3 vaccinees (Fogel et al. 1978; Cusi et al. 1993).

Table 3: Protective efficacy against rubella disease afforded by rubella vaccines during rubella outbreaks (adapted from Plotkin & Reef, 2004)

Reference	Vaccine strain	Population studied	No. vaccinees exposed	Protective efficacy
Chang et al. 1970	Cendehill	Childcare-centre, USA	32	100%
Landigran et al. 1974	Cendehill	Schoolchildren, USA	4103	93.5%
Grayston et al. 1969	HPV77-derived	Schoolchildren, China	3259	94%
Beasley et al. 1969	RA27/3	Primary schools, China	198	99.5%
Furukawa et al. 1970	RA27/3	Boys' school, Japan	24	100%
de Valk & Rebière. 1998	RA27/3	Primary school, France	119	95%
Greaves et al. 1983	NS*	High-schoolchildren, USA	>600	90%
Davis et al. 1971	Several	Institution, USA	22	100%

* NS = not stated.

Table 4: Subclinical reinfection in rubella vaccinees during rubella outbreaks: studies with serological confirmation of reinfection

Vaccine	Number of vaccinees	Time after immunization	Evidence of subclinical reinfection	Reference
HPV77	8	7 months	7 (87.5%)[a]	Portnoy et al. 1969
HPV77-derived	589	2-9 months	4.4%-12.7%	Abrutyn et al. 1970
HPV77-derived	11	17-34 months	0	Davis et al. 1971
Cendehill	15	2-3 months	10 (67%)[b]	Horstmann et al. 1970
Cendehill	4	8 months	2 (50%)	Davis et al. 1971
RA27/3	102	5 years	10 (9.8%)	Cusi et al. 1993

a Virus excretion detected in two.
b Virus excretion detected in one.

3.3.2 Correlates of protection

Specific antibodies correlate with immunity, but it has not been possible to identify a specific type and level of antibodies which are invariably correlated with absolute protection. However, rubella IgG antibodies >10 iu/ml are considered to provide protection to the majority of people (see section 2.2.3). Some studies suggest that NT antibodies provide the best measure of protection (Cusi et al. 1993), although O'Shea et al. (1994) showed that reinfection could occur in women with NT antibodies. The differences reported may be due to the use of different methods to detect NT antibodies. HI antibodies are not always associated with protection, and symptomatic and sub-clinical reinfection has occurred in patients with rubella antibodies >15iu/ml and HI titres ≥16 (Forrest et al. 1972; Enders & Knotek, 1989; Morgan-Capner, 1986; Cusi et al. 1993; Bullens et al. 2000). In a challenge study, Harcourt et al. (1980) showed that high levels of serum and NP IgA were associated with protection in RA27/3 vaccinees, but not in volunteers with naturally-acquired immunity, or those who had received other rubella vaccines.

More recent studies have suggested that antibodies to neutralizing epitopes (e.g. E1 residues 213-239), and B- and T-cell responses to the structural proteins E1 and E2, may be associated with protection (Mitchell et al. 1996, 1999). Cusi et al. (1993) also showed that vaccinees who experienced reinfection had no antibodies to E1 and E2. Studies in a mouse model demonstrated that antibodies to E1 may be essential for protection (Cusi et al. 1995), since mice given RA27/3 vaccine were protected against infection by wild-type virus, but not by recombinant E2 and C proteins. Further studies are required to confirm these results. Studies using a standard NT would also be of value, as inconsistent results have been obtained with the different NT assays used to date.

Evidence for immunological memory comes from studies which have shown a rapid secondary antibody response and high avidity IgG antibodies following vaccination of persons with naturally-acquired or vaccine-induced antibodies. Persistence of CD25+CD45RO+CD4+ T-lymphocytes also provides evidence of immunological memory (Toyoda et al. 1999). Secondary antibody responses were also seen in individuals whose rubella antibodies had declined to undetectable levels by HI, although pre-existing antibodies were usually detectable in such persons by more sensitive techniques, such as EIA (Best et al. 1980; Buimovici-Klein et al. 1980; Balfour et al. 1981; Butler et al. 1981; Serdula et al. 1984; Horstmann et al. 1985; Schiff et al. 1985; Matter et al. 1997).

3.3.3 Reinfection

Reinfection is usually subclinical and is more likely to occur in persons with vaccine-induced immunity than in those whose immunity is naturally acquired. It is not due to antigenic variants of RV (Bosma et al. 1996; Frey et al. 1998). Reinfection is defined as a significant rise in antibody concentration in a person with pre-existing antibodies. In a clinical situation, pre-existing antibodies can be confirmed by testing an earlier stored serum, but if no such serum is available, evidence of pre-existing antibody may be accepted if there are at least two previous laboratory reports of antibodies >10 iu/ml obtained by reliable techniques (not HI), or a single result of antibodies >10iu/ml obtained after documented rubella vaccination (Best et al. 1989). Rubella IgM may be detected in reinfection if a serum sample is obtained at the right time (4-6 weeks after rubella contact), and if a sensitive technique is used for its detection (O'Shea et al. 1983; Morgan-Capner et al. 1985; Best et al. 1989; Best & Enders, 2007).

Occasional case reports of symptomatic reinfection have been published (reviewed by Morgan-Capner, 1986; Bullens et al. 2000). Symptomatic reinfection has also been detected following challenge of persons who had received experimental rubella vaccines in the 1960s (Detels et al. 1969), and following challenge with unattenuated virus (Schiff et al. 1985). The occurrence of symptoms suggests that a viraemia has occurred. This would be of concern in a pregnant woman as it might lead to fetal infection. Some challenge studies have attempted to detect viraemia and virus excretion and have shown that virus excretion could be detected more frequently than viraemia (Table 5). In the six studies shown, viraemia was detected in three individuals with very low levels of pre-existing antibody; two had been challenged with the untenanted. Howell strain and one with intranasal RA27/3. Following revaccination with RA27/3, Balfour et al. (1981) also detected viraemia and virus excretion in one adult volunteer who had low levels of antibody detectable only by enhanced HI and enhanced radioimmunoassay, although no viraemia or virus excretion was detected in 11 schoolgirls with similar low

levels of vaccine-induced antibody. These studies may have failed to detect RV in some cases, as RV isolation is technically demanding and they were conducted in the 1970s and 1980s before PCR was available.

Table 5: Experimental challenge studies which included tests for viraemia and virus excretion

Study	Pre-challenge		Challenge virus and route	Viraemia	Virus excretion	Significant rise in titre	Rubella IgM detected
	Immunity	Antibody titre[a]					
Naficy et al. 1970	RA27/3 Natural	20-320 80-160	Brown, IN	0/11 0/4	0/11 0/4	2/11 2/4	NE NE
Horstmann et al.	Cendehill Natural	GMT 34.6 GMT 101	Natural Natural	NE NE	1/15 0/39	10/15 5/149	1970 NE
Chang et al. 1973 NE	Cendehill		16-128	Cendehill	NE	0/10	1/23
Balfour et al. 1981	HPV77.DE5	<8[b]	RA27/3 SC	0/11	NE	11/11	3/8
O'Shea et al. 1983	Cendehill HPV77.DE5 RA27/3 Unknown vaccine Natural Natural or vaccine	<15iu/ml <15iu/ml <15iu/ml <15iu/ml <15iu/ml <15iu/ml	RA27/3 HT, IN	0/6 0/2 0/7 1/4 0/12 0/10	1/6 0/2 0/7 2/4 0/12 1/10	6/6 2/2 7/7 4/4 8/12 3/10	1/6 1/2 0/7 2/4 0/12 0/10
Schiff et al. 1985	RA27/3	<8[c]	Howell, IN	2/5	5/5	5/5	2/5

a Titres obtained by HI.
b Nine girls had antibodies detected by enhanced HI.
c Four vaccinees had antibodies detected by latex agglutination.
NE = Not examined.
IN = Intranasal.
GMT = Geometric mean titre.
SC = Subcutaneous.
HT = High titre.

Studies of reinfection in pregnancy also suggest that a viraemia may occur in some patients, as rare cases of CRS have occurred in infants born to mothers who experienced rubella reinfection, although some had pre-existing antibody concentrations >15iu/ml (Best et al. 1989; Morgan-Capner et al. 1991; Fogel et al. 1996; Bullens et al. 2000). A small number of patients have been included in prospective studies of reinfection in pregnancy, which have shown that RV may sometimes be isolated from products of conception following TOP, or an infant born with CRS (Best et al. 1989; Morgan-Capner et al. 1991; Fogel et al. 1996). The risk of congenital infection following reinfection in the first 12 weeks of pregnancy has been estimated in the United Kingdom to be about 8%, while the risk of congenital rubella defects is probably no more than 5%, which is considerably less than the >80% risk of primary rubella during the same period of pregnancy (Morgan-Capner et al. 1991; Bullens et al. 2000; Morgan-Capner & Crowcroft, 2002). Thus, it is important to be able to use laboratory tests to distinguish reinfection from primary rubella in pregnancy (Best & Enders, 2007).

3.3.4 Immunity in the immunocompromised

Live attenuated vaccines, such as MMR and rubella, should not be given routinely to immunosuppressed persons, or persons with immunodeficiency diseases, because there is a risk of enhanced vaccine virus replication. Geiger and colleagues reported on a 16 year-old male with acute lymphoblastic leukemia in complete remission and on maintenance therapy for three months, who was immunized in error and developed acute arthritis and arthralgia in the second month post vaccination. RV was detected by PCR up to eight months after vaccination (Geiger et al. 1995). However, children with certain conditions may be vaccinated as described below.

Children with juvenile idiopathic arthritis using methotrexate, have been vaccinated with MMR with no adverse effects (Heijstek et al. 2007).

HIV-positive persons may be vaccinated, unless they have severe immunosuppression (Moss et al. 2003; UK Department of Health, 2006; Centers for Disease Control, 2006). Rubella immunization of HIV-1 infected children on anti-retroviral therapy at 15 months of age, resulted in good immune responses in those children with no immunosuppression (Lima et al. 2004). Antibody titres were inversely correlated with HIV viral load and directly correlated with CD4+ T-cell numbers, and children with moderate-severe immunosuppression had lower antibody titres.

MMR vaccine has been safely used in children with congenital T-cell defect (DiGeorge syndrome). All 13 children vaccinated responded with antibody titres not significantly different from healthy controls, and no decrease in CD4+ cells was noted (Azzari et al. 2005).

There is concern that children on chemotherapy for cancer may lose vaccine-induced immunity to various pathogens. Van Tilburg et al. (2006) conducted a systematic review of the literature concerning vaccine-induced immunity in children after cessation of chemotherapy for acute lymphocytic leukaemia. They reported that 72%-92% children had antibodies to rubella, and those without responded to revaccination. Patel et al. (2007) have recommended that children should be revaccinated with one vaccine dose at six months after completion of chemotherapy.

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The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

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