

Varicella-zoster virus infection: natural history, clinical manifestations, immunity and current and future vaccination strategies

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SUMMARY

Varicella-zoster virus (VZV) is the etiologic agent of varicella (chicken pox), a childhood exanthematic disease that develops as a result of primary infection, and zoster (shingles), caused by reactivation of the virus persisting in a latent form in the dorsal sensory ganglia. Although varicella is generally a mild self-limiting illness, in immunocompromised subjects and adults it can have a serious clinical course that can lead to permanent damage of the central nervous system. In these and in most zoster cases, treatment with anti-herpetic drugs and/or immunotherapy is necessary.

Because it is highly contagious, varicella is one of the most common exanthematic diseases. It is preventable by vaccination with an attenuated vaccine administered around the first year of age, and with a boost vaccination in school age.

This article briefly describes the natural history and pathophysiology of VZV infection and its current epidemiology and provides an overview of current and future vaccine options to protect against varicella and/or zoster.

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INTRODUCTION

Varicella-zoster virus (VZV) belongs to the Herpesviridae, large enveloped viruses with an icosahedral capsid and a double-stranded DNA genome. Herpesviruses are very common among mammals: eight have been identified in man and are transmitted in different ways from the earliest stages of life. Because of this, about 80% of the adult population has antibodies to most, if not all, human herpesviruses, apart from the human herpesvirus-8 that is less prevalent especially in Western countries (Abendroth *et al.*, 2010; Levin *et al.*, 2016).

Herpesviruses exhibit an extraordinary ability to induce persistent infections. The ways these viruses remain indefinitely in the infected individual differ. The α -herpesvirus subfamily, that includes herpes simplex virus (HSV) type-1 and -2, in addition to VZV, persists in nervous tissue. In neurons, α -herpesviruses have developed a sophisticated way of interacting with the expression of specific genes to maintain latency, absence of viral protein production, and the expression of microRNA and other viral factors that effectively counteract the host's defenses. Latency is alert, not passive, and directly managed by the virus that under conditions of cell suffering, upon endogenous and exogenous stimuli or changes in the immune status, turns off

latency-related genes to reactivate those that lead to viral replication (Baines and Pellets 2007; Baird *et al.*, 2013; Gershon *et al.*, 2012; Kennedy *et al.*, 2015; Zerboni *et al.*, 2014). VZV causes two different diseases during primary infection and reactivation: the first, varicella, typically occurs during childhood and is the consequence of exogenous infection by VZV, the second is zoster, which occurs due to reactivation of the latent virus many years, even dozens, after primary infection (Galetta *et al.*, 2015).

This article will briefly describe the natural history and pathophysiology of VZV infection, diagnosis and therapy and its epidemiology prior to the introduction of vaccination. In addition, current and future vaccination options to protect against primary infection and reactivation will be discussed.

Natural history and pathophysiology of VZV infection
Chickenpox, or varicella, is a pandemic, highly contagious rash illness transmitted by inhalation of saliva droplets dispersed in the air by subjects with acute infection or, rarely, by direct contact with skin lesions of subjects with varicella or zoster.

Because of the absence of a suitable animal model, the natural history of infection is not clearly understood. In the most accepted model, VZV at entry gives rise to a short local replication in the upper respiratory tract, in epithelial cells and the tonsils (Ku *et al.*, 2004). In these sites, it infects T cells and gives rise to a subclinical cell-associated viremia about 4-6 days after infection. During viremia, the virus is disseminated to reticuloendothelial tissues, including liver and spleen, where it further multiplies. VZV is eventually transported to the skin and mucous mem-

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branes with a second viremic phase occurring about 14 days after infection (*Figure 1*) (Abendroth *et al.*, 2010; Heininger and Seward, 2006). Memory T cells seem to have a significant role in promoting viral replication in epithelial cells during which viral gene products downregulate the IFN- α response mounted by adjacent epidermal cells (Zerboni *et al.*, 2014). Once the antiviral response is overcome, viral replication in infected keratinocytes and ensuing cell damage and inflammatory and immune response cause the formation of vesicles filled with virions. The resulting skin rash (exanthema) shows up about two weeks (10-21 days) after infection (Ku *et al.*, 2004). Contagiousness is maximum 1-2 days before the onset of a rash, when the virus spreads by droplets and aerosols from the nasopharynx, during the first 5-7 days after the appearance of rash (Abendroth *et al.*, 2010; Gershon *et al.*, 2015; Heininger and Seward, 2006; Ouwendijk *et al.*, 2015).

The rash involves maximally the trunk, with small pruritic maculopapular vesicles that spread to the neck and limbs. After 12 to 72 hours the lesions turn into pustules (similar to vesicles but containing purulent material) that often break down giving rise to scabs. Lesions do not all appear and evolve into scabs at the same time, but rather appear in waves, also involving the mucous membranes, especially in the oral cavity and tonsillar area. The pustules usually heal without leaving sequelae but, if scratched, they

can become infected by staphylococci and streptococci and leave a permanent scar. The asynchrony of lesion appearance and their poor diffusion at the distal extremities of the limbs were distinctive features from smallpox rash. As scabs form and fall off, contagiousness gradually decreases.

Varicella rash is preceded by prodromal symptoms, such as generalized malaise, nausea, loss of appetite, high fever and headache. Prodromal symptoms and lesion pain are mild in children. The disease is more serious in adolescents and adults and is particularly severe in subjects with immunological deficits due to HIV, leukemia or lymphoma, chemotherapy or steroid therapy for asthma or other diseases. It is self-healing and generally heals in one to two weeks. Complications are rare in healthy children but more common in immunodeficient infants, teenagers or adults. The most common complications are bacterial superinfection of the pustules (5% of cases), laryngitis, pneumonia (1%), thrombocytopenia and neurological problems (1-3.5 every 100,000 children under 14 years). These are much more frequent in immunodepressed subjects and include encephalomyelitis, cerebellar ataxia, arthritis, hepatitis, haemorrhagic nephritis, myocarditis, and otitis media (Ellis *et al.*, 2014; Galetta *et al.*, 2015; Gershon and Gershon, 2013; Gershon *et al.*, 2015; Moffat *et al.*, 2007; Ouwendijk *et al.*, 2015; Roderick *et al.*, 2012).

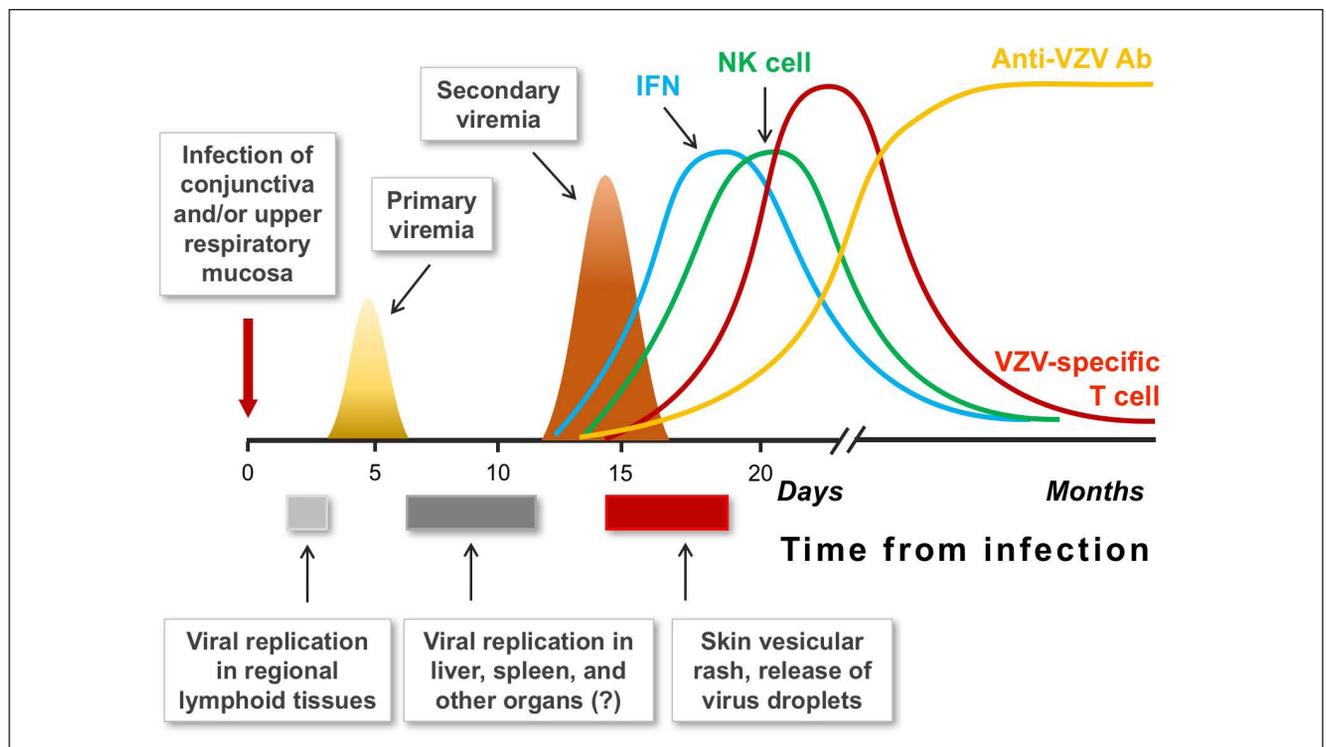


Figure 1 - Schematic representation of the VZV life cycle. Infection takes place when the virus reaches the mucosal epithelial sites and initiates local replication. This is followed by spread to tonsils and other regional lymphoid tissues. Here, VZV infects T cells and gives rise to a transient, low-grade viremia that delivers the virus to the reticular endothelial system for a second massive burst of replication. Through blood circulation, the infected T cells eventually transport the virus to the epidermal cells of the skin and mucous membranes. At these sites, VZV replicates causing the typical vesicular lesions and is released into droplets from the respiratory tract. Latency is established in innervating neurons. Immune responses to infection immediately ensue. Interferon- α and - β (IFN) produced by resident skin cells and recruited dendritic cells are the first to appear in the circulation. Natural killer (NK) cells are also activated early in infection and presumably kill infected cells. The appearance of VZV-specific T cells coincides with the resolution of skin lesions and is accompanied by sharp increases in specific IgM and IgG.

If the infection is contracted in the first two trimesters of pregnancy, VZV can be transmitted to the fetus causing the congenital varicella syndrome that occurs with skin scars, ocular defects, hypoplasia of the limbs and neurological alterations. Fetal damage often leads to miscarriage. Fetuses exposed to VZV after the fifth month in utero may develop asymptomatic chickenpox and herpes zoster early in life. If the mother develops varicella five days before to two days after delivery, the newborn can exhibit a serious form of varicella with mortality rates close to 30% (Aro-ra *et al.*, 2017; Mandelbrot, 2012; Sauerbrei and Wutzler, 2007).

Acquired immunity is permanent and reinfections are rare (Gershon and Gershon, 2013; Gershon *et al.*, 2015; Weinberg and Levin, 2010). Despite a robust immune response, VZV is not eradicated but migrates from mucous and epidermal lesions to sensory root ganglia or, in some instances, the cranial nerve where it remains latent throughout life and can reactivate to spread from the ganglion, via the sensory nerve root, to the innervated target tissue (skin, cornea, auditory canal, etc.) (Figure 1 and Figure 2).

Contrary to what was thought in the past, reactivations are frequent and occur subclinically in most cases. Consequent viral replication and the resulting production of antigens recalls immune memory and maintains immunity, thereby attenuating subsequent reactivations and al-

lowing the virus to “regenerate” by refreshing the pool of infected cells (Baird *et al.*, 2013; Cohen, 2007; Gershon *et al.*, 2012; Zerboni *et al.*, 2014; Quinlivan and Breuer, 2014). Clinically evident reactivation, or zoster, takes place in 10-20% of subjects who contracted the natural infection, with a likelihood of developing zoster increasing proportionally with age, especially after 50 years of age (Figure 2). During zoster, the virus crawls back to the dermis using nerve fibers that depart from the ganglion latency site. In the dermis and the epidermis, the virus establishes a new replication cycle with the appearance of clusters of vesicular lesions in the dermatome innervated by the nerve fiber; most commonly on the chest. Typically, a single dermatome is involved, although two or three adjacent dermatomes may be affected. Lesions are accompanied by localized pain that, in some individuals, is so intense and prolonged to require the administration of local anesthetics. In a number of cases, pain persists for over a month and is characterized by skin hypersensitivity (post-herpetic neuralgia). The incidence of zoster varies, as said, with age: this is about 4.5 total individuals/1000. At 40-50 years, it affects 3 people out of 1000, while at 80 the incidence increases to over 10 cases/1000 (Amjadi *et al.*, 2017; Bennett and Watson, 2009; Chakravarty, 2017; Gershon *et al.*, 2012; Giovanni *et al.*, 2016; Johnson *et al.*, 2015; Keating, 2016; Vrcek *et al.*, 2017; Weaver, 2009; Yawn and Gilden, 2013).

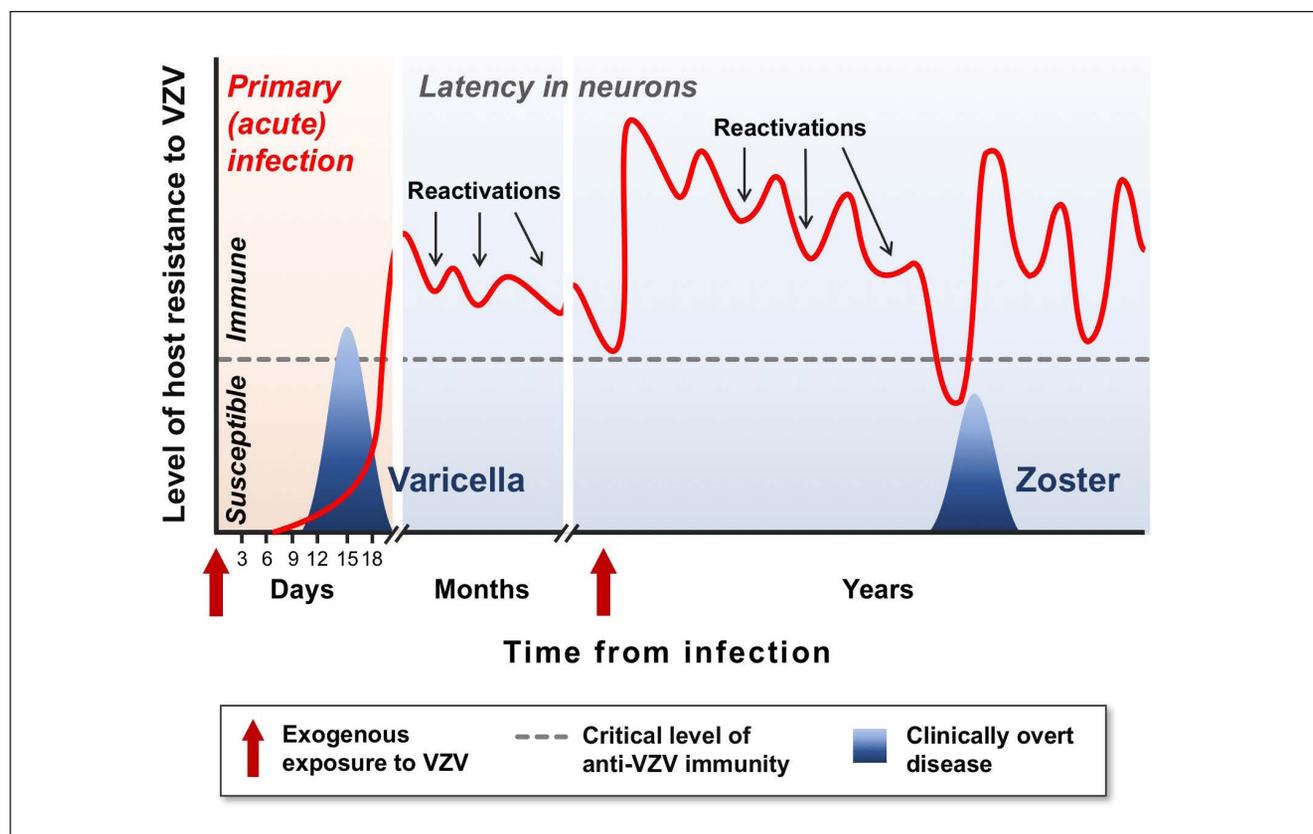


Figure 2 - Reactivation of latent VZV and new encounters with the virus maintain immunity. Following acute infection, VZV persists in neurons and periodically reactivates. According to the Hope-Simpson hypothesis, subclinical reactivations of endogenous infection and occasional external contacts with varicella keep up immunity, here indicated by the red line, thus providing long-term protection against reinfection and clinically evident reactivation. Immunity, however, declines with age. In some people, periodic reactivation can fall below the critical threshold level of anti-VZV immunity indicated by the dotted grey line, and cause zoster. Vaccines to prevent zoster are designed to boost specific immunity to avoid reactivation from trespassing the critical threshold (modified from Gershon and Gershon, 2016).

Immunity to VZV infection

Natural infection by VZV causes very early release of Interferon (IFN) type I in the blood stream, and IFN- α is also delivered locally to varicella lesions by plasmacytoid dendritic cells (Gerlini *et al.*, 2006). It is very important, but not the key innate immune mechanism, as shown by the fact that mutations affecting its entity, such as in STAT-1, have not been associated with serious cases of varicella (Hambleton *et al.*, 2013). NK cells can also be found early (*Figure 1*) (Arvin *et al.*, 1986). Although direct evidence of the role exerted by NK cells during varicella is still lacking, clinical evidence suggests that this arm of immunity is crucial. NK cells and primed CD8⁺ T cells were nearly absent from the circulation during the early phase of life-threatening primary VZV infection (Bano-*vic et al.*, 2011; Vossen *et al.*, 2005). In contrast, T lymphocyte responses to VZV are proven to be protective by several lines of evidence (Duncan and Hambleton, 2015). During varicella, T cell-mediated immunity is rarely detected until skin lesions can be seen. The detection of T cells within three days after the appearance of the varicella rash and rapid host response to primary VZV infection have long been known to be associated with milder rash and more rapid clearance of viremia in healthy subjects (Arvin *et al.*, 1986). Very recently, the ability of younger individuals to mount rapid cellular responses to VZV reactivation has been linked to protection against zoster (Weinberg *et al.*, 2017). In addition, the entity of VZV-specific CD4⁺ T cell responses was shown to inversely correlate with both the severity of disease and viremia levels (Malavige *et al.*, 2008). Conversely, the naturally occurring decrease in T cell immunity to VZV with age seems to be responsible for the occasional reactivation of VZV leading to zoster (*Figure 2*) (Miller, 1980; Levin *et al.*, 2003; Shirane *et al.*, 2013). VZV-specific CD8⁺ T cells are also elicited during natural infection (Arvin *et al.*, 1986). They exert an important protective role, as in other viral infections, but their role in recovery is controversial (Barton *et al.*, 2012; Vossen *et al.*, 2005). Recent work has correlated the levels of dysfunctional, exhausted CD8⁺ T cells in the elderly to the inability to mount an effective and timely response to VZV causing appearance of zoster (Weinberg *et al.*, 2017).

VZV infection induces robust humoral (IgM, IgG, and IgA) in addition to cellular-mediated immunological responses (*Figure 1*). Antibodies persist for a lifetime and are believed to provide protection from possible reinfections, as proven by the protective effect of VZV-specific seroprophylaxis (Morell and Barandun, 1988). However, the early production of IgG or IgM does not seem to correlate with the severity of primary clinical infection or with the appearance of zoster (Arvin *et al.*, 1986; Duncan and Hambleton, 2015). However, their rise after vaccination has been found to be an excellent marker for protection against zoster recurrent infection (Gilbert *et al.*, 2014).

Immune responses, elicited by the attenuated VZV Oka vaccine strain, are thought to be qualitatively similar to the ones induced by the wild type strain, although on a smaller scale, because the viruses only differ by very few mutations (Quinlivan *et al.*, 2014).

Laboratory diagnosis and therapy

Diagnosis is often performed only on a clinical basis but laboratory tests are still essential and used routinely in cases of disseminated infection and in atypical cases. The

Table 1 - Subjects for whom administration of anti-VZV immunoglobulin is recommended (adapted from Marin *et al.*, 2013).

Immunocompromised subjects with no specific anti-VZV immunity
Newborn infants from mothers who show signs and symptoms of varicella within 5 days before to 2 days after delivery
Hospitalized premature infants born at >28 weeks of gestation whose mothers do not exhibit specific immunity
Hospitalized newborns born at <28 weeks of gestation, weighing less than 1 Kg at birth, independently of the mother's immune status
Pregnant women with no evidence of immunity against VZV and diagnosed with recent VZV infection

virus is usually searched for by molecular tests, on skin lesion fluid, or cerebrospinal fluid in the case of central nervous system involvement. Sometimes the analysis is extended to blood and respiratory samples. Serologic diagnosis is useful to identify unprotected individuals and to distinguish primary infection from reactivation. The co-presence of IgM and IgG is indicative of recent infection or vaccination. The presence of IgG only indicates previous exposure and immunity from reinfection (Gershon *et al.*, 2015; Heininger and Seward, 2006; Mandelbrot, 2012; Sauerbrei, 2016). The absence of antibodies does not exclude infection by VZV both because they begin to be detectable by the time of rash appearance and because false-negative and false-positive results for IgM and false-negative results for IgG have been reported using validated commercial diagnostic systems (Kinno *et al.*, 2015; Wiese-Posselt *et al.*, 2017). In cases of suspected infection and in the presence of negative serological tests, the subject should be retested for antibodies within two to three weeks.

Therapy is generally only symptomatic. In children at risk of complications and, in general, in adolescents and adults, intravenous or oral administration of antiherpetic agents, such as acyclovir and derivatives (valacyclovir and famcyclovir), can be used. In some HIV-positive patients, the emergence of VZV strains resistant to acyclovir has been reported. These subjects had been treated with the drug for protracted periods of time, plausibly favoring the selection of viral variants defective or mutant for thymidine kinase, the viral enzyme necessary for the activation - by phosphorylation - of acyclovir. A drug active against acyclovir-resistant VZV is Foscarnet, an analogue of pyrophosphate also used against cytomegalovirus and HSV. Foscarnet does not require phosphorylation (Abendroth *et al.*, 2010; Gérard and Salmon-Céron, 1995; Gershon and Gershon, 2013; Schuster *et al.*, 2016).

Immunotherapy is an effective alternative to chemotherapy and is used for the subjects listed in *Table 1*. Purified immunoglobulins or hyperimmune sera containing specific anti-VZV antibodies are administered intramuscularly within 96 hours and up to 10 days after the appearance of rash. After this period, the efficacy of immunoglobulins wanes, though this is not yet fully demonstrated (Garruba and Donkers, 2013; Kim *et al.*, 2014; Marin *et al.*, 2013).

Epidemiology

Varicella was very common before the introduction of vaccination. Today, also due to poor vaccine coverage, it

is the most widespread rash illness in Italy. In the United States, there were about 4 million cases (about 1600 cases per 100,000 inhabitants), 11,000-13,000 cases of hospitalization and 100-150 deaths per year in the early 1990s (Amjadi *et al.*, 2017; Wharton, 1996; Yawn and Gilden, 2013). With the introduction of vaccination in 1995, the number of cases decreased by 79% in the 2000-2010 period compared to the pre-vaccination era and, since the introduction of a second vaccine dose, by 93% in 2012. In the same year, hospital admissions and deaths from varicella decreased by 90%. The spectacular reduction rate is due to an effective vaccine policy, which also allowed the rapid establishment of herd immunity: in the period 2010-2015, 90% of children between 19 and 35 months received a vaccine dose (83-95% in individual states); the same levels were reached among adolescents, of whom 80% received two doses of vaccine (CDC, 2017; Nguyen *et al.*, 2005). Compared to the US, the situation is much more heterogeneous in the 38 countries of the European Union: vaccination began ten years later and follows policies that differ from country to country. It is part of the routine vaccination program in three countries (Germany started in 2004, Greece in 2006, and Latvia in 2008), in a few regions of Italy (Sicily since 2003, Veneto since 2007, Apulia and Tuscany since 2010), and in certain autonomous regions of Spain (since 2006-2009), while it is recommended only for individuals at risk and immunocompromised patients in 17 countries (including Italy and Spain for the regions not mentioned above). There is no policy for the other 12 nations (Paganino *et al.*, 2015).

Different surveillance systems (non-existing in some states) and clinical case definitions make it difficult to have a clear picture of varicella incidence and complications. In one of the most comprehensive reports signed by the European Network for monitoring vaccine-preventable diseases (EUVAC.NET), about 5,500,000 cases of varicella were reported for the period 2000-2007 in the 15 countries with mandatory notification. This corresponds to an average of about 320 cases per 100,000 inhabitants. The lower incidence found compared to the United States is accounted for by a different policy in reporting cases: for example, in the period 2000-2007, Spain reported twice as many cases as Italy and Poland and over four times as many as states with no obligation to notify. Regardless of the number of cases reported, the incidence was greater in the ages of 1-4 years, as expected (2588 cases/100,000) and 4-9 years (1943/100,000) (de Boer *et al.*, 2014; EUVAC.NET, 2010; Helmuth *et al.*, 2015; Marin *et al.*, 2008; Paganino *et al.*, 2015; Yawn and Gilden, 2013).

According to data collected by the SPES network (Sorveglianza PEdiatri Sentinella) of the Italian National Health Institute (ISS), varicella has been the most frequent infectious disease in Italy, with an average of about 105,000 cases/year from 1996 to 2004. Since then, also thanks to the vaccination policy introduced in some regions, the number of cases has been steadily falling from almost 98,000 cases of 2006 to 60,000 in 2009. The main peaks of incidence were observed over the period March to May and December without any significant differences by geographical area. The most heavily affected age group is between 1 and 4 years of age, with an incidence of 7300 cases per 100,000 inhabitants, 1377/100,000 in the 5-14 age range in 2004, the year when the highest number of cases was recorded (de Boer *et al.*, 2014; ISS, 2017a).

Current and future vaccination strategies

History of the development of a successful vaccine strain

The observation that specific immune defenses developed following natural infection give lifelong immunity to subsequent reinfections suggested the idea that a vaccine could be obtained against VZV. In the mid-1960s, due to the extraordinary results achieved with vaccines against poliovirus and the fact that the spread of varicella was such that, for example in Japan, there were similar number of cases and births per year (Ozaki and Asano, 2016), a competition started between states for the production of a vaccine. At present, all vaccine strains have so far been derived from the one obtained in 1971, in Japan, by Dr Michiaki Takahashi who was the first person in the world to obtain a live and attenuated strain derived from the wild virus isolated from the vesicle fluid of an infant with varicella. The strain was named Oka from the surname of the child's family, a choice that today would not be deemed appropriate for ethical and legal issues. Attenuation was obtained with the traditional serial propagation scheme in non-human cell cultures. Since it is highly species-specific, VZV replicates only in human and monkey cells. The parental strain of Oka, however, showed a modest initial replication activity in embryonic guinea pig cells. Adaptation to these cells took place following isolation and propagation of the parental strain in human lung embryonic cells cultured at 34°C. The vaccine stock was then generated with 12 passages in guinea pig cells followed by other short passages in WI-38 and MRC-5 (diploid human cells).

Oka was used for the first time in 1974 in a Japanese clinic to successfully protect 23 children from varicella transmission by a child admitted to the same pediatric ward (Takahashi *et al.*, 1974). The vaccine, licensed by the Japanese firm Biken, was subsequently approved for use on high-risk leukemic children, again in Japan, (Gershon and Gershon, 2013), then for healthy children in Japan and Korea in 1989 and was finally approved for children over 12 months of age by the FDA in 1995 (Arnould and Mes-saudi 2017; Ozaki and Asano, 2016).

Among anti-VZV vaccines derived from Oka and marketed today, the most common are: the live attenuated, monovalent vaccines Varivax, Varilrix and Zostavax; the live attenuated, tetravalent vaccines Proquad and Priorix-Tetra that also immunize against measles, mumps, and rubella. Other vaccines comprise the subunit vaccine Shingrix (or HZ/su, recently approved), the heat-inactivated vaccine V212 (in advanced phase of experimentation), and a few others under preclinical testing. Vaccinal preparations already in use for mass vaccination or in the pipeline will be described individually in the following chapters. For the sake of simplicity and ease of comparison, *Table 2* shows their main advantages and disadvantages.

The molecular mechanisms that led to Oka attenuation are not yet well defined. It is known that its propagation on non-human cell lines has caused the accumulation of mutations along the genome, as expected, but lack of an animal model and genetic heterogeneity of vaccine preparations have hitherto prevented the definition of the contribution of individual mutations to the attenuated phenotype (Quinlivan *et al.*, 2011; Quinlivan and Breuer, 2014). Despite this, over 100 million doses of Oka

Table 2 - Pros and cons of live attenuated, inactivated or subunit vaccines against VZV.

Vaccine	Pros	Cons
Live, attenuated		
Varivax, Varilrix, Zostavax, ProQuad ^a and Priorix-Tetra	Stimulate both cellular and humoral immune response	Not recommended for subjects who are immunosuppressed due to chemotherapy, radiotherapy, steroids, neoplastic diseases, etc.
	Long-lasting immune protection	Not recommended for subjects who are immunosuppressed genetically or due to acquired diseases (HIV, tuberculosis)
	Immune response stimulated by spontaneous reactivation of vaccine strain	Risk of shedding and transmitting vaccine strain to immunodepressed subjects
		Cold chain integrity needs to be preserved in order to maintain infectivity
		Not recommended for pregnant women. Pregnancy should be avoided in the 3 months following vaccination.
		Immunity and effectiveness tend to wane over time after vaccination.
Heat-inactivated		
V212	Safe in immunodepressed subjects	Stimulates lower and less efficient immune response compared to live attenuated vaccines
	Does not require maintenance of cold chain integrity	Requires 4 doses for protective immunity, complicating compliance
Subunit		
Shingrix	Contains only one viral protein	High incidence of adverse events
	Safe in immunodepressed subjects	

^aFor tetravalent attenuated vaccines (containing also measles, mumps and rubella attenuated strains), only aspects relative to VZV are discussed.

have been administered, resulting in a dramatic fall in the incidence and mortality of VZV infection with very few adverse effects.

LIVE ATTENUATED VACCINES

Efficacy, effectiveness and duration of immune response

Live attenuated anti-VZV vaccines are excellent immunogens, therefore they all show high efficacy, defined as the ability to yield an immune response. Retrospective studies carried out on hundreds of thousands of subjects demonstrate that these vaccines induce antibody and cell-mediated response in over 95% of subjects. As far as effectiveness is concerned, or their capability of protecting against the disease, rates between 50 and 70% in reducing the risk of developing clinical manifestation of zoster have been reported, with differences depending mainly on the age of subjects. Regarding duration of the immune response, this has been seen to decline over the years, but remains at a protective level against zoster 10 years after vaccination. To obtain a long-lasting protective effect, two distant vaccine doses are recommended and now adopted by most schedules in many countries (Ansaldi *et al.*, 2016; Bennett and Watson, 2009; de Boer *et al.*, 2014; Helmuth *et al.*, 2015; Skull and Wang, 2001; Vrcek *et al.*, 2001; Wang *et al.*, 2016). Live attenuated VZV vaccine is not recommended in subjects with primary or acquired immunodeficiency (Table 2) because of the risk of developing zoster within 4-6 weeks of vaccination (Maggi *et al.*, 2015; Marin *et al.*, 2013; Oxman and Schmader, 2014). However, given the high incidence of zoster and its complications, vaccination of subjects with immunological disorders is often performed in clinical practice, offering some benefit. In this case, efficacy and effectiveness are expected to be reduced compared

to immunocompetent subjects but are still significant: reduction of the risk of developing clinical manifestation of zoster ranges between 35 and 45% in several studies, depending on the age and immunological dysfunction of subjects (Ansaldi *et al.*, 2016; Chakravarty, 2017; Garruba and Donkers, 2013; Giovanni *et al.*, 2016; Hardy *et al.*, 1991; Wang *et al.*, 2016).

Tolerability and adverse reactions to vaccination

Again, studies have been carried out on very sizeable cohorts. In most cases, only transient adverse effects to vaccination have been reported. The most common is the onset of a skin rash of variable extension within one month of vaccination, which heals spontaneously without significant sequelae. Other cases report pain, redness, and swelling at the inoculum site. Most studies report an incidence of adverse effects ranging from 2 to 4 per 10,000 vaccine doses. Case-control studies did not find any significantly increased risk of exacerbation or induction of diseases such as systemic lupus erythematosus, Guillain-Barré syndrome, multiple sclerosis, neuritis, thrombocytopenia, or vasculitis. The most serious adverse effects are those involving the central nervous system, symptoms of which may vary and include aseptic meningitis and cerebellar ataxia. These are, however, very rare: one of the most comprehensive systematic studies reports 30 neurological adverse effect cases out of 16,683 adverse events reported. In none of these, however, was the vaccine strain found in the cerebrospinal fluid of subjects (Flatt and Breuer, 2012). In general, therefore, the vaccines are well-tolerated with a low risk of mild to moderate adverse effects and very rare severe adverse reactions (Ansaldi *et al.*, 2016; Arnold & Messaoudi, 2017; De Boer *et al.*, 2014; Flatt and Breuer, 2012; Hardy *et al.*, 1991; Johnson *et al.*, 1997; Marin *et al.*, 2008; Wang *et al.*, 2016).

Persistence and reactivation of the vaccine virus

VZV Oka causes an infection that is promptly contained by the immune system and, like the wild virus, subsequently persists in the ganglia in a latent state (Gershon *et al.*, 2012; Kennedy *et al.*, 2015; Quinlivan and Breuer, 2014). Reactivation occurs periodically, almost always silently and only rarely in a clinically manifest way (Ansaldi *et al.*, 2016; Arnould and Messaoudi, 2017; de Boer *et al.*, 2014; Skull and Wang, 2001). Reactivation is, on the one hand, beneficial because it boosts immunological memory (Figure 2). On the other hand, it is a reason for concern because it could cause zoster even at a young age (Brunell and Argaw, 2000; Cimolai *et al.*, 2014; Schmid and Jumaan, 2010). It has also been argued that zoster from Oka may occur more frequently in old age due to aging of the immune system (Figure 2) (Baxter *et al.*, 2013; Cohen, 2007; Gagliardi *et al.*, 2016; Gershon *et al.*, 2015; Gershon and Gershon, 2016; Krause and Klinman, 1995; Weaver, 2009). For this reason, in some countries, such as Great Britain, vaccination against VZV is not included among pediatric vaccines and is only offered to people at risk of contracting varicella and developing complications (Ansaldi *et al.*, 2016; Gershon and Gershon, 2016). Recently, however, these concerns have been proven to be unfounded, because prospective studies in Japan, South Korea, Europe and the United States have shown that children vaccinated in the '70s and '90s have a lower risk of developing zoster than those who contracted the natural infection (Sadzot-Delvaux *et al.*, 2008; Schmid and Jumaan, 2010). Another recent study shows that the incidence of zoster in vaccinated subjects between 0 and 17 years of age was 79% lower than in subjects of the same age infected with wild-type VZV (Weinmann *et al.*, 2013). The same protective effect is obtained in subjects with reduced immune efficiency: a study on children with leukemia shows that the incidence of zoster after vaccination is 67% lower than in leukemic children who contracted natural infection (Hardy *et al.*, 1991).

Interference with vaccination

In addition to constitutive or occasional host factors discussed for each individual vaccine, common interfering factors for all anti-VZV vaccines are the presence of maternal anti-VZV antibodies and close vaccination with the measles-mumps-rubella vaccine (MMR). If anti-VZV vaccination is performed less than 30 days after MMR, subjects have a threefold higher risk of developing chickenpox compared to subjects of the same age in which the two vaccines were administered more than 30 days apart from each other. The reasons for such reduction in efficacy are not fully understood. It may be a combined effect of interfering antibodies induced by other immunogens, production of interferon and other molecules with antiviral activity (Verstraeten *et al.*, 2003), or a reduced responsiveness of the immune system to mitogens and antigens caused by measles infection (Karp *et al.*, 1996; Mina *et al.*, 2015). Conversely, the four attenuated vaccines administered at the same time do not interfere with each other as far as induction of immunity against VZV is concerned (Lapphra and Scheifele, 2009).

Varivax and Varilrix

Varivax is produced by Merck, Sharp & Dohme (MSD). The preparation, also known as Oka/MSD, was produced by propagating the original the Oka strain for another 31

passages in MRC-5 cells, then lyophilized. The preparation is resuspended in water before use. Varivax was the first to be approved: it was used in 1988 in Japan and Korea to vaccinate children from 12 months of age, then in 1995 in USA and in some European states for an analogous subject group. It is administered by subcutaneous injection in two doses, the first at 12-15 months, the second at 4-6 years. The latter can be administered from three months to over 13 years after the first dose. For unvaccinated adolescents, the two doses must be administered one to two months apart from each other (Marin *et al.*, 2008; Wang *et al.*, 2016).

Varilrix is marketed by GlaxoSmithKline (GSK), United Kingdom, Biken, Japan, and Changchun BCBT Biotechnology, China, and has a similar composition to Varivax and therefore it shares its mode of administration. Both have proven to be well-tolerated and highly immunogenic, with 97% children exhibiting antibodies when tested 7-10 years after vaccination (Johnson *et al.*, 1997). The effectiveness of the vaccine is 70-90% in protecting from infection and 90-100% in reducing severity of disease (Baxter *et al.*, 2013; Krause and Klinman, 1995). In 78% adolescents and adults, antibodies develop after the first dose, while 99% have antibodies after the second administration at four weeks (Ansaldi *et al.*, 2016; Baxter *et al.*, 2013; Krause and Klinman, 1995).

There are subjects who become infected with wild-type virus despite vaccination (breakthrough varicella). A recent review systematically examined 34 studies published from 1974 to 2016 reporting sixty such cases: most were mild, some required hospitalization for the involvement of various organs and six were fatal. Considering that each year more than 30 million doses are distributed, the risk of breakthrough varicella is very low (Leung *et al.*, 2017). Among its causes are exposure to wild-type virus before the vaccine is able to induce a protective immune response and the existence of factors that interfere with vaccinal infection and/or the induction of the immune response. Among them, the most common are: asthma, use of steroids, vaccination when less than 15 months old, errors in storage and administration of the vaccine and, as described above, vaccination against MMR less than 30 days prior to VZV vaccination (Gershon and Gershon, 2016; Lapphra and Scheifele, 2009; Verstraeten *et al.*, 2003; Wang *et al.*, 2016).

Zostavax

Zostavax consists of a lyophilized preparation of the live Oka/MSD attenuated strain grown on MRC-5 human cells. It contains at least 19,400 plaque forming units (PFU)/dose against the 1,350 PFU of Varivax. It was conceived and approved by the FDA for the prevention of zoster in 2006 (Oxman *et al.*, 2005, Levin, 2012). The initial study led to its approval, while subsequent larger studies showed that zoster risk is reduced by over 65% in subjects aged 50-59 years, about 50% in subjects aged 60-69 years and, in this group, post-herpetic neuralgia is prevented by 66.5% for those subjects in whom zoster does show up. In subjects over 70, effectiveness is reduced by roughly 37% as concerns zoster (Ansaldi *et al.*, 2016; Arnould and Messaoudi, 2017; Gagliardi *et al.*, 2016).

Although it is not recommended and largely ineffective in immunocompromised subjects, Zostavax did show some clinical benefit without serious side-effects in these patients (Del Giudice *et al.*, 2015; Flatt and Breuer, 2012;

Gagliardi *et al.*, 2016). Unfortunately, also due to the age of the vaccinated subjects, induced immunity is of limited duration, causing protection rates to fall to 20-30% 7 to 10 years after vaccination (Cook and Flaherty, 2015). The decrease in vaccine effectiveness is mainly due to the rapid decline in cell-mediated responses, which is fundamental to the restriction of reactivation. In these subjects, this is reduced by 40-50% one year after vaccination and returns to pre-vaccination levels after three years (Oxman *et al.*, 2005). A 10-year recall from the first dose is therefore necessary and recommended to protect individuals over 70 years from zoster (Levin *et al.*, 2003; Oxman *et al.*, 2005). The recall dose increases cell-mediated responses transiently and by levels inversely proportional to the age of the subject; the antibody response increases as well but also not as greatly as after natural reactivation (Arnould and Messaoudi, 2017). Notably, Zostavax-induced cell-mediated response cross-reacts with HSV antigens, probably leading to some degree of protection against the latter virus as well (Jing *et al.*, 2016). The vaccine is well tolerated and does not bring about significant adverse effects, even in older subjects (Del Giudice *et al.*, 2015; Flatt and Breuer, 2012; Gagliardi *et al.*, 2016; Levin, 2012).

ProQuad and Priorix-Tetra

ProQuad and Priorix-Tetra are made up of attenuated MMR combined with Oka and are produced, respectively, by MSD and GSK. They were approved in 2006 to vaccinate children 12 months to 12 years old with two doses, and are employed in the USA, Australia, Canada and various European countries (Marin *et al.*, 2010; Prymula *et al.*, 2014). ProQuad and Priorix-Tetra have the same MMR strains and titers of trivalent formulation but contain four and seven times more OKA compared to Varivax, respectively. The efficacy and immunogenicity of ProQuad and Priorix-Tetra against VZV are comparable to monovalent vaccines, while they are more likely to cause fever and measles-rubella rash than MMR (Leung *et al.*, 2015). Fever attacks are infrequent and are not associated with long-term problems (Arnould and Messaoudi, 2017; Flatt and Breuer, 2012). To reduce the risk of fever attacks, CDC recommends administering a first dose of MMR and Oka separately, at the same time but at different body sites, and a second dose of tetravalent vaccine (Marin *et al.*, 2010).

INACTIVATED VACCINES

V212

V212 is substantially the heat-inactivated version of Varivax and is recommended for the immunization of subjects with severe immunodepression, such as hematopoietic stem cell and bone marrow transplant patients (Hata *et al.*, 2002; Redman *et al.*, 1997). The first phase I and II studies showed good tolerability in young and immunocompetent subjects. A randomized, double-blind, MSD-funded study was completed in July 2017 and evaluated the safety and efficacy of the candidate vaccine in subjects with solid or hematologic cancer over a five-year period (Clinical trial No. NCT01254630, www.clinicaltrials.gov). The enrollment of patients started at the end of 2010 and the study estimated the incidence of zoster in 1000 patients per year over the study period and the appearance of adverse effects from day 1 to day 28 of vaccination for the four doses included in the vaccine protocol

(Table 2). The results are being evaluated and are not yet available but, given the good outcome in phase I and II studies, it is likely that V212 will prove to be effective and therefore suitable to vaccinate subjects for whom the attenuated VZV vaccines described above are not recommended.

SUBUNIT VACCINES

Shingrix

Shingrix, or HZ/su, is a subunit vaccine made up of the VZV envelope glycoprotein E (gE). This vaccine is produced by GSK and has been approved in US and Canada in October 2017, and more recently, in Europe and Japan, for the prevention of shingles in adults over 50 years of age (EC for Public Health, 2018; FDA, 2017).

VZV gE was chosen as an immunogen because it is the most abundant viral glycoprotein produced by infected cells and is one of the main targets of the immune system for the production of both specific antibodies and CD4+ T cell responses (Abendroth *et al.*, 2010; Moffat *et al.*, 2007; Weinberg and Levin, 2010). In the vaccine formulation, gE is a recombinant protein deleted of its membrane anchor and carboxy terminal domains produced in Chinese hamster ovary cells (Haumont *et al.*, 1996). To increase its efficacy at inducing cell-mediated responses, gE is combined with AS01_B, a liposome-based adjuvant consisting of a mixture of MPL (3-O-desacyl-4'-monoophosphoryl lipid A) and saponin QS-21, extracted from the plant *Quillaja saponaria*. MPL is a Toll-like receptor-4 agonist, whereas QS-21 increases the absorption and retention of antigen by dendritic cells (Coffman *et al.*, 2010; Lal *et al.*, 2013). A dose of Shingrix contains 50 µg of gE mixed in 100 µg AS01B (50 µg MPL and 50 µg QS-21). The vaccine schedule is two doses inoculated in the deltoid.

In phase I and II studies carried out on adult and elderly subjects, HZ/su showed excellent immunogenicity profiles: two doses of HZ/su vaccine induced antibody and cell-mediated responses against gE and VZV lysate higher than those induced by two doses of Varilrix in subjects of 50-70 years of age during a one-year follow-up. As with other vaccines, specific immune responses gradually decreased to half 42 months from vaccination (Leroux-Roels *et al.*, 2012). Three doses of HZ/su administered to hematopoietic cell transplant or HIV-positive patients proved to be immunogenic and did not cause major side-effects (Berkowitz *et al.*, 2015; Stadtmauer *et al.*, 2014). The product was also tested in two phase-III studies, named ZOE-50 and ZOE-70. The first involved over 15,000 subjects aged 50 divided into vaccine and placebo groups that were followed for over three years. The efficacy of the vaccine was 97.2% with a zoster incidence of 0.3/1,000 people/year in vaccine versus placebo 9.1 (Lal *et al.*, 2015).

In ZOE-70, 13,900 subjects with an average age of 75.6 years were enrolled, divided into two equal groups and followed for 3.7 years. In the vaccine group, the efficacy was 89.8%, with very few differences between subjects under and over eighty years. As in ZOE-50, the risk of zoster was considerably reduced in the vaccine group compared to the placebo group (0.9 vs. 9.2/1000 people/year) (Cunningham *et al.*, 2016; Cunningham and Heineman, 2017).

The very good news has been offset by the incidence of adverse effects: in ZOE-50, 85.1% of vaccinees reported solicited local symptoms (pain, redness and swelling) at the injection site, 66% reported solicited systemic symp-

toms (fatigue, fever, gastrointestinal symptoms, headache and myalgia) and 11.4% of subjects over 50 years of age reported solicited systemic symptoms of grade 3, defined as significant pain that prevented normal daily activity (Arnold and Messaoudi, 2017; Lai *et al.*, 2015).

CONCLUSIONS

The pathway VZV takes to replicate and spread within the host organism, how it persists and the multifarious clinical manifestations induced by primary infection and reactivation make the virus an extremely interesting model, also from a microbiological point of view. Oka, the attenuated strain and founder of today's VZV vaccines, is no less interesting. The mutations and the molecular mechanisms that have led to a marked change in phenotype are not known but attenuation is quite stable, millions of individuals have been vaccinated with the Oka strain with minimal adverse effects and no reversion to the wild phenotype has been reported.

Unfortunately, VZV does not raise the same interest as regards public health. Varicella is considered a benign disease with no sequelae provided that the immune system is functional and efficient and the infection is contracted at school age. However, the age of encounter with varicella and the average age of the Western population is rapidly rising, and so is the risk of developing zoster, which is painful and not easy to control, even therapeutically. From this point of view, the VZV vaccine policies adopted by many countries are hard to agree with. Italy is perhaps the most obvious example where, due to a curiously disparate regional policy, there are regions where vaccination coverage is down to an embarrassing 10% and others where it is close to 80%. Even the latter figure is, in any case, far from the goals of the recently announced National Vaccine Plan (ISS, 2017b). Hopefully, the release of inactivated vaccines for older people and greater awareness of the mid- to long-term consequences of primary infection will favor wider vaccine coverage also for VZV. This will help varicella infection no longer be the leader in the disgraceful list of the most common rash infections in childhood.

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