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Rubella virus-associated chronic inflammation in primary immunodeficiency diseases

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Purpose of review
The aim of this article is to summarize recent data on rubella virus (RuV) vaccine in chronic inflammation focusing on granulomas in individuals with primary immunodeficiencies (PIDs).

Recent findings
The live attenuated RuV vaccine has been recently associated with cutaneous and visceral granulomas in children with various PIDs. RuV vaccine strain can persist for decades subclinically in currently unknown body site(s) before emerging in granulomas. Histologically, RuV is predominately localized in M2 macrophages in the granuloma centers. Multiple mutations accumulate during persistence resulting in emergence of immunodeficiency-related vaccine-derived rubella viruses (iVDRVs) with altered immunological, replication, and persistence properties. Viral RNA was detected in granuloma biopsies and nasopharyngeal secretions and infectious virus were isolated from the granuloma lesions. The risk of iVDRV transmissibility to contacts needs to be evaluated. Several broad-spectrum antiviral drugs have been tested recently but did not provide significant clinical improvement. Hematopoietic stem cell transplantation remains the only reliable option for curing chronic RuV-associated granulomas in PIDs.

Summary
Persistence of vaccine-derived RuVs appears to be a crucial factor in a significant proportion of granulomatous disease in PIDs. RuV testing of granulomas in PID individuals might help with case management.

Keywords
chronic inflammation, granuloma, immunodeficiency-related vaccine-derived rubella viruses, primary immunodeficiency, rubella virus

INTRODUCTION

Recent advances in next-generation sequencing have led to the identification of a considerable number of primary immunodeficiency diseases (PIDs), which now comprise 406 distinct disorders associated with 430 genetic defects of the immune system [1]. Although each individual disorder is rare, collectively PID disorders are not uncommon, and at least 1/1200, or 6.5 million, individuals are currently living with PIDs [2]. Cutaneous granuloma, a serious complication in individuals with diverse PIDs, has been long thought to be largely because of immune dysregulation and therapy has focused on immune suppression [3,4]. The purpose of this review is to highlight recent findings of the association of vaccine-derived rubella virus (RuV) persistence in the inflamed tissues of PID patients and granulomas.

Prevalence of granulomas in patients with primary immunodeficiency diseases

A granuloma is a compact immunological structure, which concentrates macrophages, lymphocytes, and signaling molecules around persisting inflammatory triggers, both infectious and noninfectious [5]. This structure provides the opportunity for activation of immune effector cells that may limit infection, kill the pathogen, and then repair tissue injury. Granulomas can occur in different tissues and may be self-limited or progress to a chronic inflammatory disorder if the antigenic trigger is not eliminated. Granulomas have been described commonly in

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KEY POINTS

- There is a strong association between persistence of vaccine-derived RuVs and granulomas in immunodeficient individuals, but a causal link between rubella vaccine virus and granuloma formation has not been confirmed in prospective studies.
- Ongoing replication and evolution of vaccine-derived RuVs during persistence in PID individuals resulted in mutated viruses with altered biological properties.
- Effective treatment for most rubella-associated persistent granulomas is not now available.

children with PIDs and can be a presenting sign of PIDs [6,7]. Until recently, PID granulomas were considered to be sterile because no microorganisms had been consistently detected [3,4].

Recently, the prevalence of granulomas in individuals with PID was determined using two data sources: MarketScan database of US national healthcare claims and a PID patient disease registry, United States Immunodeficiency Network (USIDNET) [8]. Skin granuloma was the most common type, but other organs were also involved. The proportion with granulomas was similar across age groups for the MarketScan population (0.8–1.7%). In the USIDNET registry, the proportion whoever had granulomas ranged from 2 to 9%, with the lowest proportion in PID patients aged 0–19 years and highest proportion in those aged 35–44 years.

Overall, the estimated granuloma prevalence in PID patients was 1–4% comprising between 65 000 and 260 000 individuals worldwide.

ASSOCIATION BETWEEN RUBELLA VIRUS VACCINE AND GRANULOMA

The first evidence for the association between RuV vaccine and granulomas was obtained by deep sequencing of samples from in a three-case series of children with PID [9]. The RA27/3 vaccine sequences were detected in granuloma lesions but not in the normal skin of the same individuals. The association between RuV and granulomas in skin lesions in PID was further confirmed in a larger blinded study ($n = 19$) by using a different detection method (fluorescent immunohistochemical staining for the RuV capsid protein) and a different sample type (formalin-fixed paraffin-embedded tissue slides) covering a broad spectrum of PIDs [10]. Table 1 summarizes data on the identification of RuV antigen and/or RNA in a wide spectrum of PIDs with granulomas of unknown cause for published [9,10,11–14,15▪,16] and unpublished cases (Perelygina, personal communication).

Table 1. Defection of RuV in granulomas of unknown cause in PID patients

PID type	RuV pos	RuV neg	Total
AT	17	3	20
ADA–SCID, SCID unknown gene	7	7	14
CID	9	3	12
CVID	6	4	10
NBS	6	2	8
RAG1 and RAG2 deficiency	5	2	7
XLA	2	1	3
DiGeorge syndrome (22q11.2 deletion)	2	1	3
CHH	2		2
Ligase IV deficiency	2		2
PGM3 deficiency		2	2
X-SCID (IL2-receptor deficiency)	1		1
NEMO deficiency syndrome		1	1
Marden–Walker syndrome	1		1
McKusic syndrome	1		1
ARTEMIS (DCLRE1C deficiency)	1		1
TAP-1 deficiency	1		1
MHC Class II deficiency	1		1
WHIM syndrome	1		1
Coronin 1A deficiency	1		1
Total	66	26	92
% RuV positive			71.7

ADA, Adenosine deaminase; ARTEMIS -Artemis (protein name, not abbreviation); AT, Ataxia telangiectasia; CHH, cartilage hair hypoplasia; CID, combined immune deficiency; CVID, common variable immune deficiency; DCLRE1C - DNA Cross-Link Repair 1C; MHC - major histocompatibility complex; NBS, Nijmegen breakage syndrome; PGM3 Phosphoglucomutase 3; RAG, Recombination activating gene; RuV, rubella virus; SCID, severe combined immunodeficiency; TAP-1, Transporter 1; WHIM, Warts, Hypogammaglobulinemia, Immunodeficiency, and Myelokathexis syndrome; XLA, X-linked agammaglobulinemia; X-SCID, X-linked severe combined immunodeficiency.

Approximately 70% of cases, who largely had prominent T-cell defects with concomitant antibody deficiency, had granulomas positive for RuV. Many cases shared a diagnosis of a DNA repair disorder [13]. We are currently evaluating different granuloma-associated conditions in both in PID patients and immunologically normal individuals for the presence of RuV in granulomatous lesions.

RUBELLA VIRUS: BASIC VIROLOGY

RuV is a small, enveloped virus belonging to genus *Rubivius*, which has been moved in 2019 from the *Togaviridae* family to the newly created *Matonaviridae* family. The RuV genome is a 9.7-kb linear single-stranded RNA of positive polarity, which encodes for three structural proteins (envelope glycoproteins E1 and E2, and capsid protein C) and two nonstructural replicase proteins p150 and p90 [17].

The capsid protein serves as a package for genomic RNA forming nucleocapsid. The nucleocapsid is surrounded by an envelope, which is decorated by spikes consisting of E1 and E2 heterodimers. These heterodimers facilitate RuV entry and are targets for neutralizing antibody. RuV is transmitted via direct or droplet contact with respiratory secretions and is highly infectious (estimated basic reproduction number $R_0 = 5–7$) [18]. Nasopharyngeal mucosal epithelia appear to be the portal of entry and primary site of virus replication and shedding.

RuV is divided into two clades, Clade 1 and 2, which were subdivided into 10 and 3 genotypes, respectively. Clades and genotypes were identified by sequence analysis. Only 4 genotypes 1E, 1J, 1G and 2B are currently circulating worldwide with 1E and 2B being most frequently detected [19]. RuV does not interfere significantly with host cell metabolism and is not cytocidal in many cell types, resulting in the establishment of persistent infections [20,21]. Although acute rubella is a mild and often subclinical disease, persistent rubella infections can lead to chronic diseases, which were predominately associated with immune-mediated pathologies. In postnatal infections, RuV can establish persistent infections in immune-privileged body sites leading to a spectrum of clinical manifestations including encephalitis, chronic uveitis, and chronic arthritis [22–25]. RuV can also persist in developing fetal organs causing multiple birth defects [collectively known as congenital rubella syndrome (CRS)] [26,27].

RUBELLA VACCINE

To prevent CRS, several live-attenuated rubella vaccines have been developed. The RA27/3 vaccine strain originated from a Clade 1, genotype 1a RuV isolated from a CRS affected fetus in 1961 [28]. RA27/3 is commonly used in combination with other vaccines, such as measles, measles–mumps (MMR), or measles–mumps–varicella. RA27/3 has been in use in the United States and many countries worldwide for the last 40 years. The vaccine is efficacious, safe, and induces long-lasting immunity [28]. To prevent rubella and CRS, 168 countries offer rubella vaccine with a 69% worldwide coverage for rubella vaccination (www.who.int/news-room/fact-sheets/detail/rubella). Rubella and CRS has been eliminated from the United States since 2004 and was declared eliminated in the Americas in 2015 (www.paho.org).

Live-attenuated virus vaccines are contraindicated for severely immunocompromised persons (e.g., from hematologic tumors, chemotherapy, long-term immunosuppressive therapy, and persons with primary or acquired immunodeficiency) because it can lead to a severe disease. According to Advisory Committee on Immunization Practices General Best Practice Guidelines on Immunizations, the assessment of severe immunosuppression is often based on the CD4+ T cell counts (https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html). Unfortunately, in several PID disorders, T cells can be dysfunctional whereas CD4+ cell counts remain within normal limits complicating PID diagnosis and risk stratification. Furthermore, a large proportion of PID individuals are diagnosed after 1 year of age, when MMR is often given.

HISTOPATHOLOGICAL FEATURES OF RUBELLA VIRUS-ASSOCIATED GRANULOMAS

The severity of the RuV-associated granulomatous inflammation can vary from a few superficial cutaneous plaques or nonulcerated nodules located predominately on face and limbs to deep ulcerated lesions with necrosis covering large areas and leading to tissue destruction [29]. In addition to skin, RuV antigen can be found in granulomas in multiple visceral organs including lung, spleen, kidney, lymph nodes, bone marrow, and liver [12,13].

Histologically, RuV-associated granulomas are predominantly sarcoidal epithelioid type consisting of M2 type (CD68+/CD206+ and CD68+/CD163+) macrophages harboring RuV antigen at the granuloma center surrounded by lymphocytes (Fig. 1) [10]. Many viruses utilize M2 macrophages for virus replication and dissemination in tissues [30]. M2 macrophages are involved in tissue repair and persist in chronic inflammatory conditions presenting attractive long-term reservoir for virus persistence. In addition to macrophages, RuV can occasionally be detected in epidermal keratinocytes; the epidermal skin layer becomes damaged and ulcers occur [10].

Both noncaseating granulomas and caseating granulomas with necrotic center may be present in the same lesion although noncaseating granulomas usually predominate [29]. RuV positive multinucleated giant cells (macrophage syncytia or Langhans cells) can also be seen in some lesions. A distinctive feature of chronic cutaneous granulomas in PID is the predominance of CD8+ over CD4+ T cells [6,31]. CD4+ T cells have been shown to play a critical role in resolution of *Mycobacterium*-induced granulomas in tuberculosis [32]. It has yet to be determined whether insufficient CD4+ cells contribute to the inability of individuals with PID to resolve RuV associated granulomas.

The onset of RuV positive inflammatory lesions varied considerably between patents, from two months to 14 years (average two years) after receiving MMR [13]. The timing between vaccination and

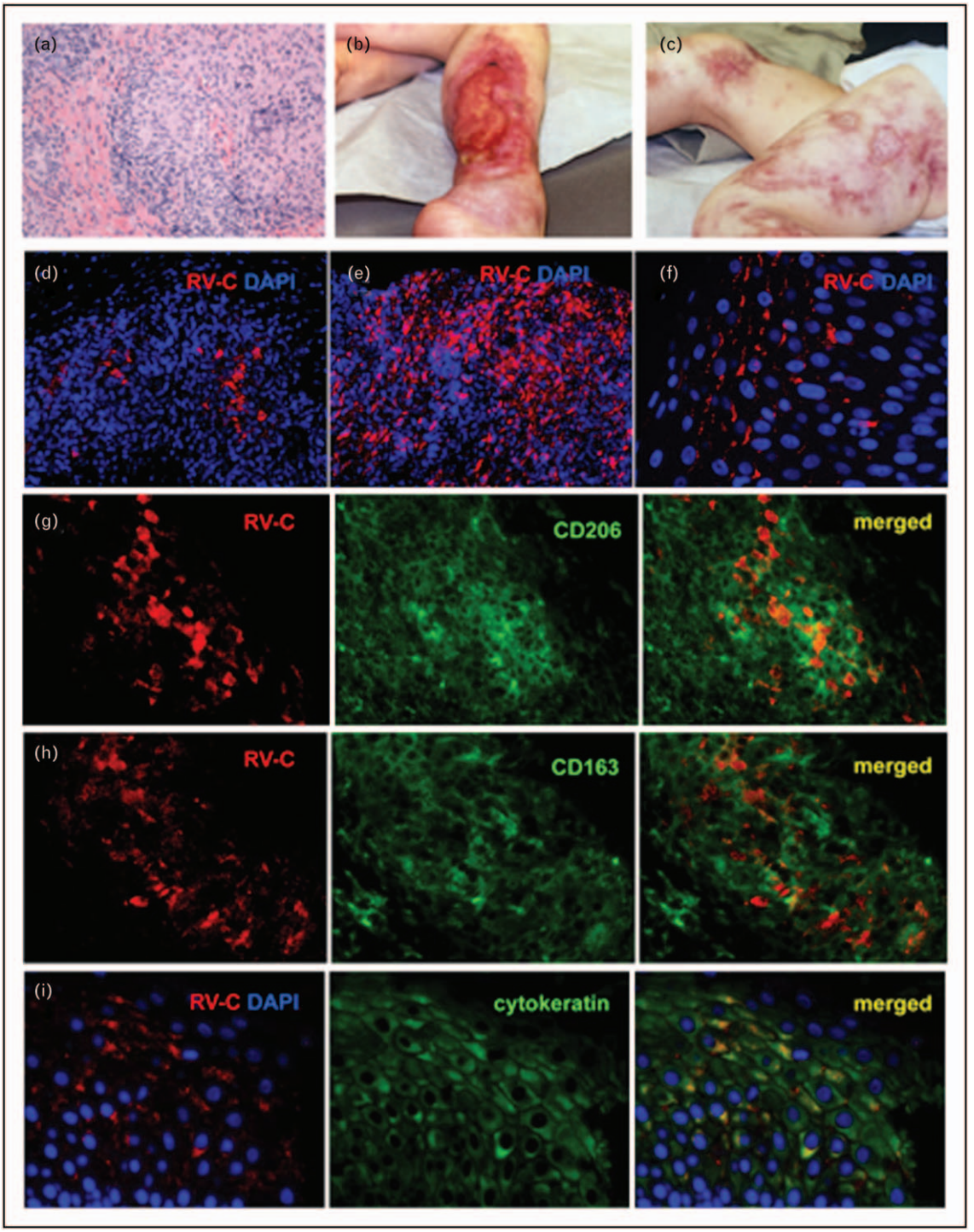

FIGURE 1. Cutaneous granulomas in PID patients. (a) Hematoxylin and eosin staining of a cutaneous granuloma from case 1. A well-formed granuloma is centrally located. (b, c) Cutaneous skin lesions from case 3. Acute and chronic ulcers are observed. (d–f) Distribution of infected cells in skin samples of PID patients. Histological immunofluorescent staining showing focal (d, case 1) or widespread (e, case 2) distribution of RuV capsid in granulomas and focal capsid localization in the epidermis (f, case 5). Activation status of macrophages in granulomas (g–i, case 6). Double immunofluorescent staining of granulomas with RuV capsid antibody (red) and M2 macrophage specific antibodies, CD206 (g, green) or CD163 (h, green). (i) RuV antigen expression in the suprabasal cell layer of skin epidermis (case 1). Double immunofluorescent staining with RuV capsid antibody (red) and keratinocyte specific antibody (cytokeratin, green). Nuclei were counterstained with DAPI, 4′,6-diamidino-2-phenylindole. Previously published in Ref. [8].

granuloma development likely depends on the magnitude of immune system dysfunction and additional host factors, such as the receipt of systemic immunosuppressive therapies.

EVOLUTION OF RUBELLA VIRUS VACCINE IN PRIMARY IMMUNODEFICIENCY DISEASE PATIENTS

Vaccine-derived RuVs can persist for decades in PID patients and the longer the persistence lasts, the more viral mutations accumulate. To date, six full-length or near full-length sequences of RuV genomic RNA from skin granuloma biopsies and from nasopharyngeal (NP) secretions of one PID patient have been published [9,10,15▪]. All sequences were derivatives of the RA27/3 vaccine strain (Fig. 2) with multiple nucleotide and amino acid substitutions and, therefore, they were designated immunodeficiency-related vaccine-derived rubella viruses (iVDRVs). It is unknown whether any of these substitutions are back mutations as attenuating mutations of the RA27/3 vaccine have not been characterized. A positive linear relationship between the number of mutations in iVDRV genomes and times after vaccination strongly indicate ongoing replication and evolution of persisting vaccine viruses in PID patients [15▪]. An overall rate of sequence evolution was estimated to be 1.8×10^{-3} nt substitutions/site/year, or 18 nt substitutions/genome/year, which is within the observed range for many RNA viruses.

PUBLIC HEALTH-RELATED ISSUES

There are several important questions from a public health standpoint: Are persisting iVDRVs infectious? Can iVDRVs be shed and transmitted to nonimmune contacts? Can iVDRVs cause CRS in nonimmune pregnant women? Is the vaccine-induced immune response protective against iVDRVs?

Some of the questions have been recently addressed. Infectious iVDRVs viruses were recovered from four out of five cutaneous granuloma biopsies, but no virus was detected in a swab from the lesion surface by reverse-transcriptase-polymerase-chain-reaction in one of those individuals [15▪]. RuV is a respiratory virus, which is predominantly shed into nasopharynx. iVDRV RNA was found in two out of five sequential NP swabs in one granuloma case out of three tested, but infectious virus was not detected [15▪]. The frequency and levels of virus shedding into the NP cavity and onto the lesion surface, as well as transmissibility of iVDRVs to nonimmune contacts has yet to be determined in a large study group.

Reduced immune pressure by the defective immune system in PIDs may be responsible for the emergence of iVDRV mutant viruses capable of low-level, decades-long persistence. Most amino acid mutations in iVDRVs occurred in the structural proteins, including mutations in the neutralizing epitopes of the E1 envelope glycoprotein and CD8+ cytotoxic T lymphocyte epitopes of the capsid protein [10,15▪]. The data on poor neutralization of iVDRV strains by sera from vaccinated healthy individuals raise the concern that some of these mutant viruses may be poorly recognized by the rubella vaccine-induced immunity. Importantly, multiple mutations have resulted in altered biological properties of the iVDRV strains compared with the parental RA27/3 vaccine strain [15▪]. The iVDRV strains were less cytopathic in cell culture, produced lower amounts of viral RNA, proteins, and infectious virions and, unlike RA27/3, can persist in primary cultures of fibroblasts, presumably the initial target cells following vaccination. Unfortunately, the lack of available animal models makes it difficult to evaluate iVDRV pathogenic properties *in vivo*.

PROGNOSTIC SEROLOGICAL MARKERS FOR GRANULOMAS

Persisting rubella immunoglobulin M and very high levels of RuV-neutralizing antibodies were found in PID patients with RuV-associated granulomas [15▪]. It remains to be determined whether these are serological markers for RuV persistence and/or predict an appearance of granulomas in vaccinated individuals.

CAUSAL RELATIONSHIP BETWEEN RUBELLA VIRUS AND GRANULOMAS

Establishment of casual relationships between persisting viruses and chronic diseases has always been problematic, especially for viruses that persist subclinically before causing disease [33]. It is even more problematic, when a disease is immune-mediated and not a result of direct virus cytopathology. Nonetheless, most of Hill's epidemiological criteria for causation [34,35] have been met for the causal link between RuV vaccine and granuloma development in PID individuals. RuV is present in 70% of granulomas in a broad range of PIDs (*strength of association*). The association between RuV and granuloma has been demonstrated by multiple laboratories in different countries using different study groups, different types of samples and by different study designs (*consistency*). Two other live-attenuated viruses in MMR, measles and mumps, have never been detected in RuV-positive granulomas; RuV is the only infectious agent detected in the lesions by NextGen

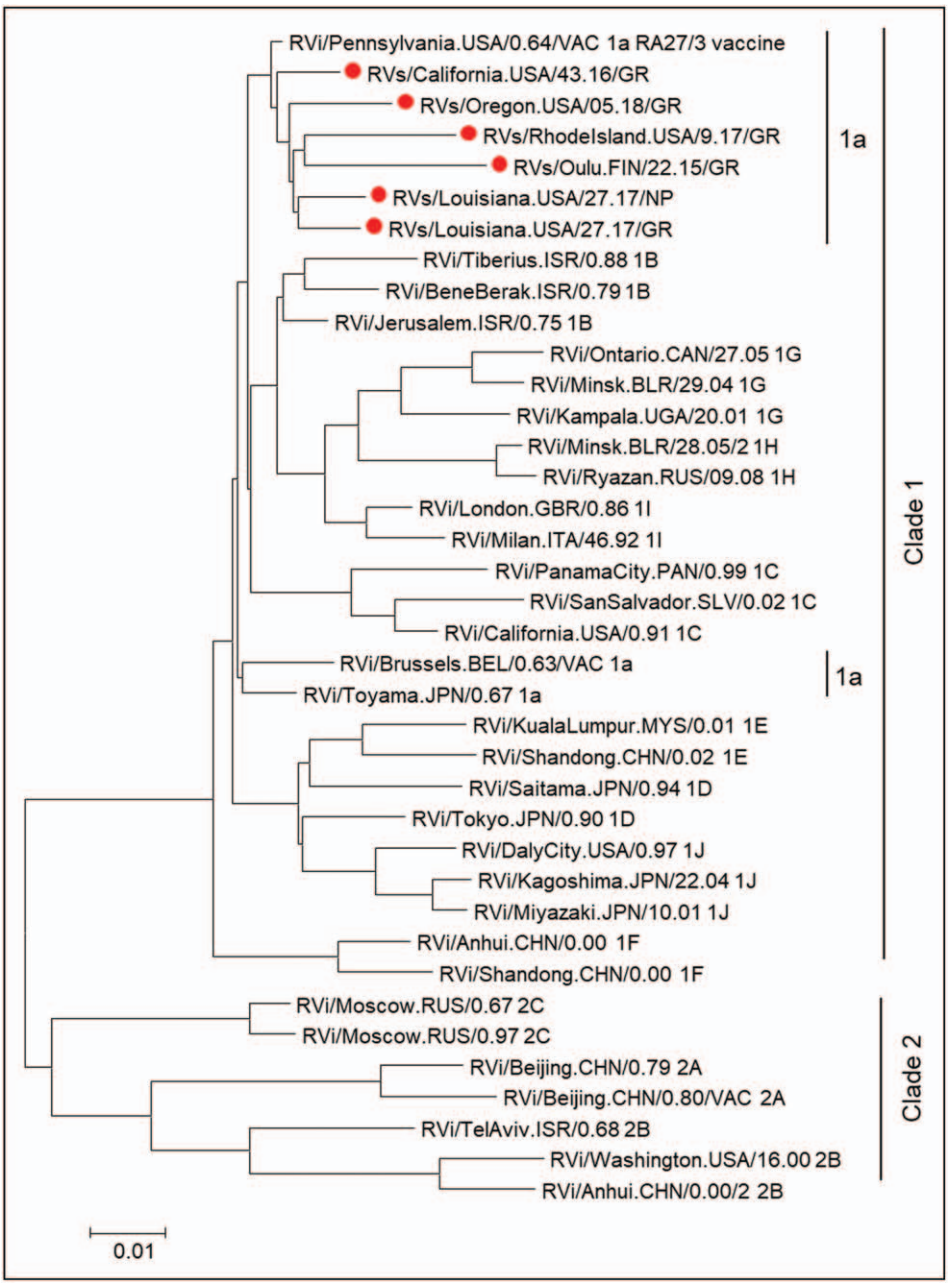

FIGURE 2. Phylogenetic tree of iVDRV. The genetic relationships between the consensus genome sequences from each original granuloma sample and the whole genomes of the WHO reference viruses were inferred using the maximum likelihood method in molecular evolutionary genetics analysis 7. All taxa are labeled with WHO names with iVDRV sequences marked with red dots. The genetic distances were computed using the maximum composite likelihood method. The scale bar indicates the number of base substitutions per site. RA27/3 and iVDRVs represent a separate branch on the tree with RA27/3 being basal. Previously published in Ref. [15▪].

sequencing (*specificity*). RuV is present in cutaneous granuloma lesions but absent in healthy skin (*a biologic gradient*). MMR vaccination precedes the granuloma development (*temporality*). Infectious iVDRVs have been isolated from granulomas and, histologically, rubella antigen has been found in macrophages in the middle of granulomas, where the granuloma causative agent is expected to be localized (*biological plausibility*). RuV vaccine persistence has been associated with other inflammatory diseases, such as uveitis, encephalitis, and arthritis (*analogy*). Nevertheless, prospective natural history studies might provide additional strong evidence of causality.

TREATMENT STRATEGIES

No effective specific therapy is currently available to cure RuV infections. The drugs with known broad antiviral properties, nitazoxanide, ribavirin and interferon, have been largely unsuccessful for treatments of patients with RuV positive granulomas [13,29,36,37]. Intravenous immunoglobulin (IVIG) therapy does not eliminate persisting RuV but may provide moderate improvement and may prevent systemic virus spread. Unfortunately, the levels of RuV neutralizing antibody in immunoglobulin preparations are unknown as well as the role of neutralizing antibody in resolution of RuV associated inflammation. Antibody-dependent enhancement of RuV disease has never been seen as a concern, but since RuV was found in macrophages in granuloma lesions, there is a theoretical potential of IVIG supplementation to amplify the infection. Current therapy for PID granulomas has focused on immune suppression, which may reduce tissue inflammation but are mostly ineffective in resolving granulomatous disease [38–40]. Caution must be given to the use of systemic steroid drugs, as it may lead to more severe rubella systemic infection. Hematopoietic stem cell transplantation is the only known effective treatment, which usually leads to complete remission. Several immunomodulatory drugs have been recently evaluated (rapamycin, rituximab, infliximab, interleukin-2) but provided only a moderate effect in the limited number of patients [13,29].

CONCLUSION

The strong association between persistence of vaccine-derived RuVs and development of granulomatous disease in individuals with various PIDs has been recently established. Chronic lesions of unknown cause in such patients should be investigated for the presence of RuV, which may impact proper diagnosis and consideration of treatment strategies for this condition. Currently used granuloma treatments are only moderately effective with exception of hematopoietic stem cell transplantation, which is not feasible for all PID patients. Identifying the precise mechanisms that contribute to long-term asymptomatic persistence of RuVs and recognizing the risk factors that trigger the development of RuV-associated granulomas will be critical for the development of more effective targeted strategies for granuloma treatments in persons with PID.

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Conflicts of interest

There are no conflicts of interest.

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