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BRIEF REPORT

Cutaneous and Visceral Chronic Granulomatous Disease Triggered by a Rubella Virus Vaccine Strain in Children With Primary Immunodeficiencies

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Persistence of rubella live vaccine has been associated with chronic skin granuloma in 3 children with primary immunodeficiency. We describe 6 additional children with these findings, including 1 with visceral extension to the spleen.

Keywords. rubella; granuloma; skin; spleen; vaccine.

Patients with primary immunodeficiency (PID) are susceptible to severe and/or persistent infections, frequently associated with opportunistic microorganisms [1]. PID is also associated with immunopathological disorders, including granulomatous diseases, characterized by the accumulation of mononuclear epithelioid histiocytes, giant cells, and T lymphocytes in various tissues. Granulomas are classically associated with microbial triggers, but attempts to identify causal microorganisms or antigens are frequently unsuccessful, and granulomas are therefore by default considered a consequence of immune dysregulation [2]. These processes have been described in a large group of primary immunodeficiencies, such as chronic granulomatous disease, common variable immunodeficiency, various T-cell immunodeficiencies including cartilage hair hypoplasia [3] or ataxia-telangiectasia, and also hypomorphic recombination activating gene (*RAG*) deficiencies [4]. We have recently reported 3 cases of extensive and persistent skin granuloma triggered by rubella virus (RV) live vaccine in the context of primary T-cell immunodeficiencies [5]. Here, we strengthen these findings by reporting 6 additional cases, 1 presenting with visceral involvement of RV-associated granuloma.

PATIENTS AND METHODS

All patients presented similar cutaneous extensive and persistent cutaneous granuloma (Table 1) in the context of PID (ataxia-telangiectasia (n = 5), hypomorphic *RAG* deficiency (n = 2), activated phosphoinositide 3-kinase δ syndrome (n = 1), and combined immunodeficiency of unknown cause (n = 1). All patients received 1 or 2 injections of measles, mumps, and rubella vaccine during childhood. Skin granuloma developed 2–145 months after the first immunization (Table 1) In all patients, skin biopsy results confirmed the diagnosis of "nonsarcoidosis granuloma," corresponding mainly to histiocyte and minor lymphocyte infiltration (Supplementary Figure S2). No infectious agent was identified by conventional methods (data not shown). Progressive multivisceral granuloma developed in 1 patient (see Case Report).

Case Report (Patient 5)

Combined immunodeficiency with profound T-cell lymphopenia and hypogammaglobulinemia was diagnosed in patient 5 at age 9 years in the context of repeated bronchopulmonary infections. Anti-infectious prophylaxis was prescribed, with trimethoprim-sulfamethoxazole, azithromycin, and intravenous immunoglobulin replacement therapy. Two heterozygous missense mutations in *RAG1* (p.H375D and p.Y562C) were subsequently identified, encoding for *RAG1,* which is involved in VDJ recombination [6]. This patient had also had progressive confluent subcutaneous ulcerative purplish nodules on his right arm (Supplementary Figure S2, panel 1) and leg when he was 2½ years old, which persisted, despite local anti-infectious and anti-inflammatory therapies. Progressive hepatosplenomegaly had also been present since childhood and peripheral thrombocytopenia developed in the patient's teenage years, concomitant with worsening of skin lesions and right leg lymphedema. When the patient was 15 years old, thoracoabdominal computed tomography revealed mediastinal adenomegalies, bronchiectasis involving right middle and inferior lobes, diffuse nodular and micronodular lesions in the lung parenchyma (Supplementary Figure S2, panel 2), and heterogeneous liver and spleen. Magnetic resonance imaging of the right ankle adjacent to cutaneous lesions showed diffuse micronodular

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Table 1. Status of the Patients

Patient	Sex	Diagnosis	CD3 Cell Count (Nadir, Cells/μL) or Other Cellular Immunodeficiency Markers	Hypogammaglobulinemia	Age at Vaccination, mo	Age at Onset of Skin Lesions, mo	Rubella RT-PCR and IHC Results (Sample Tissue; Age at Sampling, mo)
1[a]	F	Ataxia-telangiectasia	Low (1150)	Present	16	18	PCR positive (skin granuloma; 43); PCR positive (skin granuloma; 103); IHC positive (skin granuloma; 103); PCR positive (lymphomatous lymph node; 107)
2[a]	M	Activated phosphoinositide 3-kinase δ syndrome[b]	Low CD4 naive cell count	Present	13 and 67	132	PCR positive (skin granuloma 131 and 180); IHC positive (skin granuloma; 180); PCR negative (healthy skin; 195)
3[a]	F	Ataxia-telangiectasia	Low (672)	Present	17 and 28	33	PCR positive (skin granuloma; 59); PCR negative (healthy skin; 59)
4	F	Undefined combined immunodeficiency	Low CD4 naive and NK cell counts	Present	NA	NA	PCR positive (skin granuloma; 20)
5	M	*RAG1* deficiency	Low (540)	Present	13 and 18	30	PCR positive (skin granuloma; 179); PCR and IHC positive (spleen granuloma; 198); PCR positive (skin granuloma; 200); PCR negative (healthy skin; 200)
6	F	Ataxia-telangiectasia	Low CD4 naive cell count	Present	13	45	PCR positive (skin granuloma; 78)
7	F	*RAG2* deficiency	Low (418)	Present	18	21	PCR positive (skin granuloma; 35); PCR negative (healthy skin; 35)
8	M	Ataxia-telangiectasia	NA	Present	8 and 13	24	PCR positive (skin granuloma; 53)
9	M	Ataxia-telangiectasia	Low (773)	Present	11 and 88	156	PCR positive (skin granuloma; 196)

Abbreviations: IHC, immunohistochemistry for rubella virus; mo, months; NA, not available; NK, natural killer; PCR, polymerase chain reaction; RT, reverse-transcriptase.

[a] Cases report published in [5].

[b] Previously suspected to be Simpson-Golabi-Behmel syndrome.

intraosseous lesions enhanced after gadolinium injection (Supplementary Figure S2, panel 2). Skin biopsy samples revealed an infiltrate of mononuclear epithelioid histiocytes and few giant cells (not shown). Results of direct examination and culture of biopsy samples were negative. The granulomatous nature of the pathogenic multivisceral process was confirmed by histological analysis of needle liver and lung biopsy samples and spleen specimen obtained after subtotal splenectomy when the patient was 16 years old, as part of prehematopoietic stem cell transplantation assessment. Examination of spleen biopsy samples disclosed numerous and coalescent nonnecrotic epithelioid granulomas throughout the red and the white pulp (Supplementary Figure S2, panels 3 and 4). Reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry to detect RV were performed on granulomatous skin and spleen biopsy samples.

Samples and Extraction of Nucleic Acids

Biopsies of granulomatous skin, spleen, and healthy skin and blood fractions (serum, plasma, and peripheral blood mononuclear cell pellet) were performed (Table 1 and Supplementary Table S1). Total RNA was extracted from tissue biopsy samples according to the TRIzol procedure (Thermo Fisher Scientific) and purified with the RNeasy Mini columns (Qiagen), treated with DNAse 1. Nucleic acids were extracted from liquid fractions (serum and plasma) using the QIAamp cador Pathogen Mini Kit (Qiagen).

RV Detection With Immunohistochemistry, RT-PCR and Sequence Analysis

Details on RT-PCR and sequence alignment with the Wistar 27/3 attenuated RV strain (accession No. FJ211588) and immunochemistry performed using a monoclonal anti-rubella capsid antibody (Abcam ab34749) are provided as supplementary material.

RESULTS

RT-PCR Detection of the RV Vaccine Strain Wistar 27/3 in Skin and Spleen Granuloma

Guided by the results obtained for the first 3 patients [5], RV detection was conducted using RT-PCR with primer pair 8669F/SPR8 in the 6 additional patients with PID and

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granuloma of unknown cause. Positive results were recorded in skin granuloma for all patients, including when repeated sampling was performed (patient 5, Table 1). Amplicons sequence showed highest identity and greatest similarity with the RV vaccine strain Wistar 27/3 in each patient (Supplementary Figure S1 and Tables S2–S4). Healthy skin of patients, when sampled concomitantly, was negative (Table 1), supporting the association of RV with granuloma development. A spleen specimen from patient 5 was also found RT-PCR positive for RV strain Wistar 27/3. All blood fractions (serum, plasma, and peripheral blood mononuclear cell pellet) were negative for this patient from 2014 to 2015 (Supplementary Table S1).

Detection of the RV Capsid by Immunohistochemistry in Spleen Granuloma

Immunohistochemistry showed the presence of a strong cytoplasmic signal with an antibody directed against RV capsid in numerous macrophages of spleen epithelioid granuloma (Supplementary Figure S2, panel 4), and no signal was detected among control epithelioid granulomas in nonimmunodeficient patients or in biopsy samples from a patient with tuberculous granulomas (not shown). Skin and liver samples were not available for immunohistochemistry.

DISCUSSION

We reported previously that a RV vaccine strain can persist for years in children with PID and is associated with skin granuloma. Here we report on a larger series of patients with granuloma and primary T-cell immunodeficiency, and all patients tested so far are RV positive (n = 9) (Table 1). RV sequences form "patient-specific" clusters (Supplementary Figure S1, patients 2 and 5), in favor of the hypothesis of RV vaccine strain persistence and progressive intrahost evolution, rather than recent reinfections from vaccinated contacts. Findings in patient 5, a child with hypomorphic *RAG1* deficiency and combined immunodeficiency, show that granuloma associated to a RV vaccine strain can be a visceral process, because RV capsid proteins were also found in spleen granuloma. Although not sampled in the context of standard management of the patient, granulomatous formations found in bone, lung, and liver may also be reasonably attributed to the same process. Profound T-cell deficiency was identified in this patient, which might contribute to RV dissemination and granuloma development and persistence.

Vaccination with an attenuated microorganism may lead to a generalized infection in the case of sterilizing immunity deficiency. Live vaccines are therefore contraindicated in patients with primary T-cell immunodeficiencies, yet their diagnosis may be delayed until after the period of infancy immunizations [1], and chronic infections associated with live vaccine have been described in this setting [7]. The full spectrum of RV-associated diseases after vaccination as well as the type of immunodeficiency concerned (T cells, B cells, innate antiviral immunity) remains to be precisely defined. Long-term persistence of wild type rubella strain has been proposed in patients with arthropathy, but a causal relationship was never formally established [8]. More recently, a well-documented case of RV-associated uveitis was described in a 73-year-old patient without contemporary RV viremia [9].

Mutations in the *RAG1* or *RAG2* genes in humans are associated with a broad spectrum of clinical phenotypes [10], and association with granuloma, autoimmunity, and autoantibodies have been reported ([4, 10, 11]). Our results suggest that granuloma formation in these patients could also be associated with a protracted response to xenoantigens [5].

In the cases reported herein, RV antigens were evidenced in granuloma macrophages, which is consistent with its distribution in persistently infected patients with congenital disseminated rubella [12]. This macrophage tropism supports a role as a trigger of granuloma formation. We also strengthen the very strong association of RV with granulomatous disease, because all tested lesions in 9 children presenting with at least skin granuloma, in the context of demonstrated or suspected PID, were positive for RV, whereas results were negative for healthy skin from the patients and controls ([5] and Table 1). To our knowledge, RV is the first virus described as strongly associated with granuloma development in the context of immunodeficiency and must be added to the list of the potential triggers of chronic granuloma.

In conclusion, we have demonstrated the persistence for up to 15 years of a RV vaccine strain in a series of patients with PID and its association with the development of skin and spleen granulomas. Persistent expression of RV antigens in macrophage within granulomas makes it the likely trigger for granuloma formation.

Supplementary Data

Supplementary materials are available at http://academic.oup.com/cid. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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