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Disclosure of potential conflict of interest: E. de Schryver declares being employed at Ghent University Hospital. R. Valenta declares a grant from the Austrian Science Fund and grants/grants pending as well as providing consultancy to Biomay AG, Thermofisher, and Fresenius Medical Care. I. Mittermann declares a grant from the Austrian Science Fund. R. P. Schleimer has received research support from the National Institutes of Health; has received consultancy fees from Intersect ENT, GlaxoSmithKline, Allakos, Aurasense, Merck, BioMarck, Sanofi, AstraZeneca/MedImmune, and Genentech; and has stock/stock options in Allakos, Aurasense, and BioMarck. B. K. Tan declares grants from the National Institutes of Health. P. Gevaert declares a grant from Fonds Wetenschappelijk Onderzoek and providing consultancy to and receiving travel support from various sources. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online May 6, 2016.
http://dx.doi.org/10.1016/j.jaci.2016.03.040

Immunologic defects in severe mucocutaneous HSV-2 infections: Response to IFN-γ therapy

To the Editor:

Herpes viruses such as herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella zoster virus cause vesicular lesions of the oral mucosa and skin, but can also affect other organs.[1] After acute infection, the virus resides in a latent state and reactivates when the host immune response is impaired. Although most herpes infections are self-limiting, immunosuppression predisposes to reactivation and severity.[2] Reactivations of herpes virus infections have been reported in patients with primary immunodeficiencies, and these studies demonstrated that innate pattern recognition is crucial for protection.[3] In some patients, the reactivation of HSV-2 may be very frequent and severe, and difficult to treat with antiviral therapy. The mechanism behind this increased susceptibility to severe recurrent skin infections with herpes viruses remains unknown in most patients. In this study, we identified defective IFN-γ production due to specific defects in double-stranded RNA (dsRNA) recognition in 3 patients with severe recurrent HSV-2 skin eruptions. Standard antiviral therapy failed, but all patients responded with impressive resolution of their symptoms upon substitution with recombinant IFN-γ.

The first patient is a 39-year-old woman who suffered from recurrent HSV-2 skin infections on the right side of her face and ear since she was 3 months old, with frequencies varying from monthly to weekly. Treatment with intravenous acyclovir and prophylaxis with valacyclovir had little effect on the symptoms, despite no identification of antiviral resistance of the virus. No herpes infections were documented in her parents; the patient has no siblings or children (see Fig E1 and this article's Online Repository available at www.jacionline.org).

The second patient is a 59-year-old woman who suffered from a severe infection with vesicular lesions when she was 11 years old and recurrent vesicular lesions on her left buttock every 3 to 4 weeks since she was 41 years old. HSV-2 was identified in the vesicular lesions. Prophylaxis with valacyclovir lost its efficacy because of acquired resistance. Her mother and grandmother have also reported recurrent herpetiform lesions, 1 sister (III.7) had recurrent genital herpes infections, and a niece (IV.2) had a severe varicella zoster virus infection at the age of 10 years (Fig E1, *B*).

The third patient is a 49-year-old woman who suffered from severe recurrent oropharyngeal HSV infections since the age of 16 years, treated with valacyclovir prophylaxis. No viral infections were documented in the family, but her son suffered from *Staphylococcus aureus* endocarditis at age 9 years (Fig E1, *C*).

Immunologic assessment did not reveal defects of T-, B-, or natural killer cells (see Table E1 in this article's Online Repository at www.jacionline.org). Cytokine production capacity of

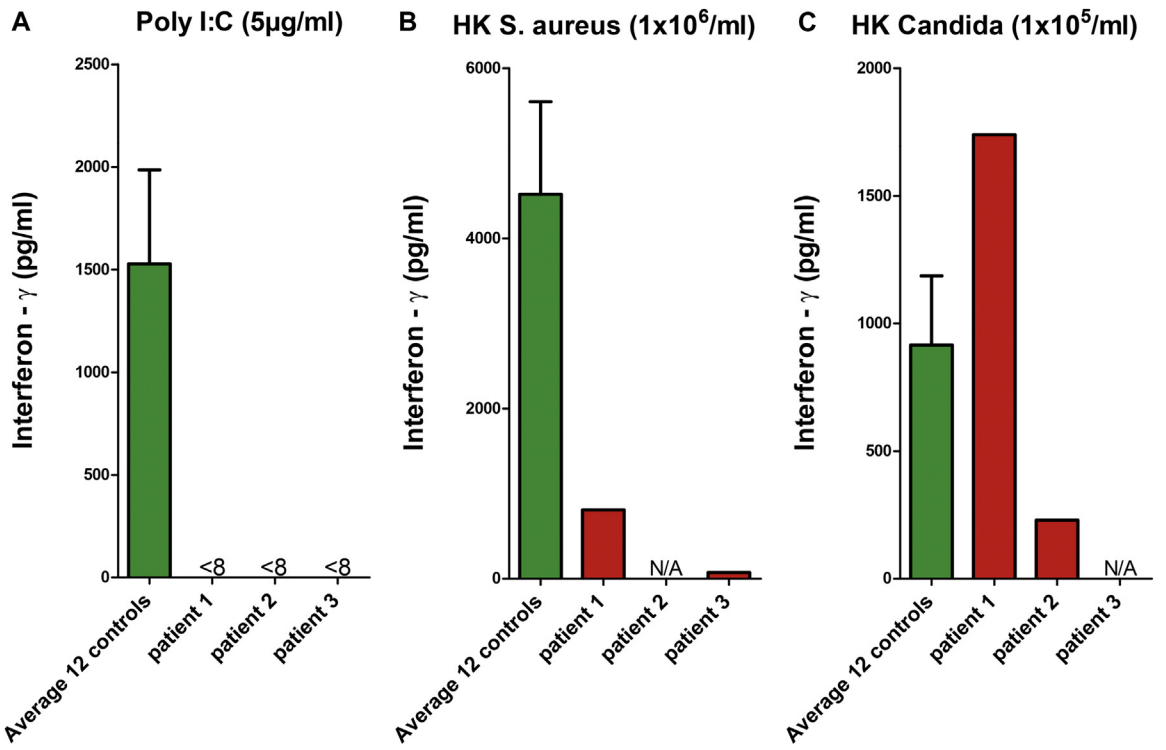

FIG 1. Immunological defect in patients with HSV compared with controls. IFN-γ levels (y-axis) were measured after *in vitro* stimulations with polyI:C(A) **(A)**, *Staphylococcus aureus* **(B)**, or *Candida albicans* **(C)**. *In vitro* stimulations were performed of PBMCs from patients and compared with 12 controls (x-axis). After 48 hours of stimulation, IFN-γ production was measured by an ELISA. The normal *C albicans*–induced IFN-γ response in patients 1 and 2 implicates the specific polyI:C and *S aureus* defect in our patients. *HK,* Heat killed.

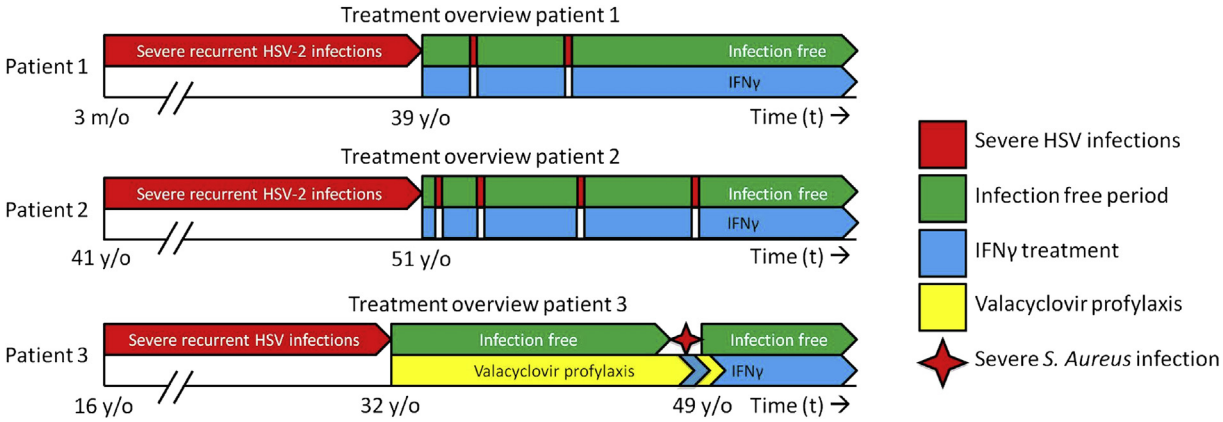

FIG 2. Treatment overview for IFN-γ in our patients shows the recurrent HSV infections over time (*t*). Severe HSV infections are significantly reduced during the periods with IFN-γ therapy, and relapsed during the periods when treatment was stopped. Mild HSV infections were sporadically observed during periods of rIFN-γ treatment. Patient 3 shows the different infections over time (*t*). HSV infections similar to Steven-Johnson syndrome starting at age 16 years. After 4 severe episodes, she started valacyclovir prophylaxis for 17 years. At age 49 years, she suffered from a severe *S aureus* infection and rIFN-γ treatment was started. Six weeks later, she stopped valacyclovir prophylaxis, and she remained infection-free since.

PBMCs of patients with HSV-2 infections was normal upon stimulation with LPS, Pam3Cys, whole bacteria, or fungi. In contrast, polyI:C (TLR3 ligand) stimulations of PBMCs isolated from all 3 patients resulted in complete absence of IFN-γ production (Fig 1, *A*). PBMCs from patient 3 also showed defective IFN-γ production upon stimulation with *S aureus* (Fig 1, *B*), whereas the response to *Candida albicans* was normal (Fig 1, *C*). Production of type I interferons (IFN-α and IFN-β) by cells isolated from patient 1 and patient 2 was 30% to 50% of that of normal volunteers (no data were available from patient 3).

The functional data were supplemented by whole-exome sequencing that was performed in all 3 index patients similar to

previous studies[4] (detailed methods of the immunologic and genetic testing are given in this article's Online Repository available at www.jacionline.org).

Exome sequencing did not provide rare variants in the same gene in all 3 patients. However, several rare variants in possible candidate genes were identified, with the most interesting candidates being as follows: patient 1, *HDAC5* (p.E836Q and p.P886H) and *NLRX1* (p.R860Q); patient 2, *ZBTB25* (p.S7G) and *IFIT1* (p.R77W); and patient 3, *POL3RB* (p.P162A) and *POLR3E* (p.R671Q). The only variants for which full segregation with the disease could be provided were the compound heterozygous *HDAC5* variants in patient 1, and the heterozygous *ZBTB25* variant in the family of patient 2 (Fig E1, *B*), which was present in all affected family members, but absent in other healthy siblings. The histone deacetylase 5 (*HDAC5*) was reported to regulate the inflammatory response in macrophages and influence anti–HSV-1 immunity.[5] The Zinc finger and BTB domain-containing 25 (*ZBTB25*) has been proposed as a T-cell–enriched transcription factor that negatively regulates activation of the nuclear factor of activated T cells.[6] Details on all candidate variants are available in the Results section and Tables E2-E5 in this article's Online Repository available at www.jacionline.org.

Because of the defect identified in IFN-γ production, we assessed the clinical effect of replacement therapy with recombinant (r) IFN-γ (Immukine 3 times a week 100 μg subcutaneous injection). Treatment of patient 1 with rIFN-γ in combination with oral valacyclovir resulted in a strong decrease in reactivations from severe episodes every 4 to 6 weeks to 1 mild episode a year. She twice attempted to stop the treatment followed by an immediate HSV-2 reactivation within 1 month. The patient has been treated with chronic administration of rIFN-γ for the past 3 years (Fig 2). Similarly, treatment of patient 2 with rIFN-γ decreased the frequency and severity of HSV-2 episodes from every 3 to 4 weeks with valacyclovir alone, to an average of 1 mild episode every 9 months. Treatment was stopped 4 times in 3 years (because of the adverse effects of rIFN-γ, mild flu-like symptoms, and inconvenience of subcutaneous injection), which was followed by immediate severe HSV-2 reactivation (Fig 2). Patient 3 started rIFN-γ therapy to suppress the illness that arose from *S aureus* infection and abscesses on her left buttock. She recovered, and tolerated the treatment without any adverse effects. Six weeks after the start of rIFN-γ, she stopped valacyclovir prophylaxis. Two months after stopping valacyclovir, she developed a mild herpes labialis on her lower lip, which normally, according to the patient, would progress to a severe herpes stomatitis. Instead, the lesion resolved uneventfully and the patient has remained disease-free (Fig 2).

Recurrent herpes encephalitis is associated with defective viral dsRNA recognition due to defects in the TLR3 pattern recognition pathway.[3] Our 3 patients with severe recurrent HSV mucocutaneous reactivations displayed functional defects in recognition of the dsRNA-like ligand polyI:C, resulting in defective IFN-γ responses. Natural killer and T-lymphocyte numbers were normal, although we cannot exclude functional defects in their antiviral function due to the IFN-γ deficiency. Initially registered for the treatment of chronic granulomatous disease,[7] rIFN-γ has been proposed as adjunctive therapy in patients with other infections.[8,9] In our patients, rIFN-γ induced a significant improvement in their clinical symptoms. When patients 1 and 2 attempted to discontinue IFN-γ, they promptly displayed HSV-2 reactivations, indicating that the improvement in symptoms was truly due to rIFN-γ administration.

In conclusion, we described 3 patients with frequent and severe mucocutaneous reactivations of HSV-2, in whom a specific IFN-γ defect upon stimulation with dsRNA ligands was identified. Interestingly, none of the patients or their family members reported invasive infections such as herpes encephalitis. IFN-γ replacement therapy resulted in significant amelioration in all 3 patients, and we therefore propose that IFN-γ production capacity should be assessed in patients with severe recurrent HSV-2 infections, and in case of defective production, replacement therapy with rIFN-γ should be considered.

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This study was supported by a grant from the Nijmegen Institute for Molecular Life Sciences (to A.H.). F.L.V., A.H. (grant no. 91612095), M.A.L. (grant no. 86313008), and C.G. (grant no. 91614043) were supported by Veni grants of the Netherlands Organization for Scientific Research. J.A.V. was supported by a European Research Council Starting Grant (grant no. 281964). R.L. and M.A.H. were supported by the Virgo consortium, funded by the Dutch government, and by the Netherlands Genomics Initiative. F.J.K. (grant no. 82002018) and M.G.N. (grant no. 91810610) were supported by Vici grants from the Netherlands Organization for Scientific Research. M.G.N. was supported by a European Research Council Consolidator Grant (grant no. 310372).

Disclosure of potential conflict of interest: F. L. van den Veerdonk, M. A. Langereis, C. Gilissen, F. J. van Kuppeveld, and A. Hoischen have received grants from the Netherlands Organization for Scientific Research. R. van der Lee and M. A. Huynen have received a grant from Virgo consortium, funded by the Dutch government, and by the Netherlands Genomics Initiative. J. A. Veltman has received a grant from the European Research Council (grant no. 281964). M. G. Netea has received grants from the Netherlands Organization for Scientific Research and the European Research Council (grant no. 310372). The rest of the authors declare that they have no relevant conflicts of interest.

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Available online April 7, 2016.
http://dx.doi.org/10.1016/j.jaci.2016.02.025

IL-1β enhances inflammatory T_H2 differentiation

To the Editor:

Type 2 immune responses are critically dependent on the canonical cytokines IL-4 and IL-13, 2 related cytokines that both use IL-4Rα for signaling. These mediators have both overlapping and independent functions, with IL-4 involved in the initiation of T_H2 differentiation and immunoglobulin class switching and IL-13 in T_H2 inflammatory responses. Hallmarks of infections associated with type 2 immune responses are the infiltration of affected tissues by helper T cells, eosinophils, and basophils, activation of macrophages, smooth muscle and tissue remodeling, and elevated levels of IgE.[1]

We have previously reported that IL-1β strikingly enhances CD4 T-cell survival, antigen-driven expansion, differentiation, and cytokine *in vivo* production.[2,3] To investigate the impact of IL-1β on T_H2 differentiation in greater detail, pigeon cytochrome c (PCC)-specific 5C.C7 CD4 T cells were cultured under T_H2-polarizing conditions. Anti–IL-1α/β or IL-1Ra (anakinra) was included in control cultures to generate T_H2 cells in the absence of IL-1 signaling for comparison with those exposed to IL-1β. IL-4 production was reduced in T_H2 cells primed with IL-1β compared with the anti–IL-1 and anakinra groups; in contrast, IL-13 production was dramatically increased among cells primed with IL-1β (Fig 1, *A*). IL-1R1 was also detected on more than 50% of T_H2 cells at 72 hours and maintained at higher levels on T_H2 IL-1β cells as compared with T_H2 cells treated with anti–IL-1 at 96 hours (Fig 1, *B*). To assess the contribution of IL-1R expression to this pattern of cytokine production, T cells were cultured for 2.5 days with anti–IL-1α/β or IL-1β, sorted for IL-1R1, and then placed back in their original culture conditions for 2 days before analysis (Fig 1, *C*). The data suggest that cells acquiring expression of IL-1R during early T_H2 differentiation are especially susceptible to adopting a dominant IL-13–producing phenotype when exposed to IL-1 at this time. IL-5 expression showed similar enhancement in the IL-1R+, IL-1 group (Fig 1, *D*). Similar results were obtained using wild-type and other TCR-Tg CD4+ T cells, suggesting that the phenomenon was not restricted to a single antigen or TcR (data not shown).

To assess their phenotypic stability, both T_H2 groups differentiated *in vitro* were sorted for IL-1R1 and either reprimed *in vitro* under neutral, T_H1, or T_H2 conditions or transferred *in vivo* into mice rechallenged intranasally: Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org shows that independently of the secondary condition, the original phenotypes were stably maintained.

Myd88 and nuclear factor kappa B (NF-κB) are involved in the IL-1R1 canonical signaling pathway. To analyze their contributions in our system, we cultured OT-II, OT-II IL-1R1$^{-/-}$, or OT-II Myd88$^{-/-}$ T cells together with wild-type antigen-presenting cells. Fig E1, *C*, shows that IL-1β–dependent enhancement of IL-13 production required both IL-1R1 and Myd88 expression on the responding CD4 T cells but not on antigen-presenting cells (data not shown). To test the contribution of NF-κB, T_H2 cells ± anti–IL-1α/β were treated with an NF-κB activation inhibitor. No effect was observed with anti–IL-1, but IL-13 expression was significantly diminished and IL-4 production enhanced in the IL-1β group, indicating a contribution of the IL-1R/NF-κB pathway to the cytokine production phenotype we observe after IL-1β exposure (Fig E1, *D*).

Chromatin immunoprecipitation analysis of Il13, Il4, and IFN-γ promoter regions was carried out on both T_H2 groups using antibody to the activating AcH3K27 modification (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org).[4] Il13 HSII and Il13 HSIII, but not Il13 or Il4 HSIII, showed a clear enhanced immunoprecipitation in the "IL-1β, IL-1R+" group. Thus, T_H2 priming in the presence of IL-1β causes the promoter and second intron of IL-13 to become more transcriptionally active.

Microarray (not shown) and RT-PCR analysis of both T_H2 groups showed that PTH, IL-13, IL-5, Ccl17, Timd2, Slc15a3, Slc2a6, and Nts were more highly expressed in the "IL-1β, IL-1R+" group and IL-4, IL-10, and Myo6 were more highly expressed in the "anti–IL-1, IL-1R−" group. Parathyroid hormone (PTH) showed the greatest enhancement in the "IL-1β IL-1R+" group. Its expression has previously been reported in activated T_H2 cells but not in T_H1 cells (Fig E2, *B*).[5] We confirmed PTH secretion by ELISA and measured biologically active PTH via cyclic adenosine monophosphate functional assay (Fig E2, *C* and *D*).

T_H2 differentiation is central to cell recruitment, induction of inflammation, and mucus production in the lungs.[6] Using PCC-specific 5C.C7 CD4+ T cells, lymphocytes from the anti-IL-1–treated and the IL-1β–exposed groups were sorted for IL-1R1, adoptively transferred into wild-type mice, and the mice challenged intranasally with antigen + anakinra only to avoid any potential secondary effect of endogenous IL-1β (Fig 2, *A*). The number of eosinophils in the bronchoalveolar lavage (BAL) and in the lungs was significantly greater in mice that received IL-1R+ cells primed with IL-1β. These differences were maintained when the mice were challenged 13 weeks after transfer (Fig 2, *B* and *D*). Periodic acid-Schiff staining of lung sections shows greater peribronchial infiltration of inflammatory cells, goblet cells metaplasia, and striking enhancement of mucus production (magenta) in the"IL-1β, IL-1R+" group, with an average histopathology score significantly higher than that of controls (Fig 2, *C*, *E*, and *F*).

To examine the possible role of IL-1β during an *in vivo* house dust mite (HDM) T_H2 allergic response, wild-type mice were sensitized intranasally with HDM + anakinra or IL-1β, and rechallenged 7 days later for 5 consecutive days (Fig 2, *G*).[7]