

Serological evidence that activation of ubiquitous human herpesvirus-6 (HHV-6) plays a role in chronic idiopathic/spontaneous urticaria (CIU)

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Summary

Acute infection with viral pathogens in the herpesviridae family can trigger acute urticaria, and reactivation of herpesviridae is associated with cutaneous urticarial-like syndromes such as drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DRESS). Reactivation of latent herpesviridae has not been studied systematically in chronic idiopathic/spontaneous urticaria (CIU). This review proposes that CIU is an inflammatory disorder with autoimmune features (termed 'CVU' for chronic viral urticaria), based on serology consistent with the hypothesis that reactivation of a latent herpesvirus or -viruses may play a role in CIU. Serology obtained from a cohort of omalizumab (Xolair)-dependent patients with severe CIU was consistent with previous HHV-6 infection, persistent viral gene expression and replication. CIU patients also exhibited serological evidence of increased immune response to HHV-4 (Epstein-Barr virus, or EBV) but not all CIU patients were infected with EBV. These observations, combined with case reports of CIU response to anti-viral therapy, suggest that HHV-6, possibly interacting with HHV-4 in cutaneous tissues, is a candidate for further prospective study as a co-factor in CIU.

Keywords: chronic urticaria, DRESS, herpesviridae, omalizumab, Th2 cytokine, virokin, virome

Introduction

Chronic idiopathic/spontaneous urticaria (CIU) appears to involve signalling through immunoglobulin (Ig)E receptors, as it responds to blockade of the IgE receptor by a humanized monoclonal antibody omalizumab (Xolair), but CIU also has features of an autoimmune disease based upon the presence of autoreactive immunoglobulin and correlation with other syndromes, such as autoimmune thyroid disease [1,2]. Although autoantibodies against the IgE receptor and related molecules are present in many patients with CIU, it has not been possible to demonstrate that these antibodies are present in all cases of CIU or that antibody levels correlate with disease activity. Thus, it is possible that autoreactive antibodies in CIU might be a useful diagnostic factor for CIU, rather than the primary cause of the disorder. Other observations demonstrate that the skin in CIU has a T helper type 2 (Th2) cytokine and inflammatory signature that might play a role in the disease and response to omalizumab independently or in conjunction with autoantibodies [3,4]. Although CIU responds to omalizumab it

does not alter the underlying disease process, as CIU symptoms often return with discontinuation of therapy [5,6].

Acute urticaria has similar lesions to CIU, but lasts for less than 6–8 weeks and is associated significantly with acute herpesvirus infection, although the role of herpesviruses or anti-viral therapy has not been studied systematically in CIU [7–9]. In other human autoimmune diseases, such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE), human herpesviruses, particularly human herpesvirus 4 and 6 (HHV-4 and HHV-6), have been suggested based on serological studies to play a role as co-factors in inflammation and associated autoimmune disease [9,10]. For example, HHV-4 plays a pathogenic role in the cutaneous syndrome of scleroderma, in which the virus directly infects dermal macrophages and fibroblasts and modulates innate immunity [11]. HHV-6 also infects thyroid tissues in patients with autoimmune thyroiditis, but not healthy controls [12].

In its latent phase, HHV-6 virus resides primarily in CD4 helper lymphocytes, and is unusual because it inserts

into the host genome, so that endogenous genomes can continue to express genes in the absence of free viral genomes or genome replication [13]. Importantly, recent clinical human studies and animal models of HHV-6 confirm that viral gene expression from integrated genomic copies of HHV-6 drive persistent inflammatory cytokine expression in neuroepithelial tissues [14–17]. Reactivation of epithelial HHV-6, as well as HHV-4 (EBV), also plays a role in Th2 cytokine-mediated syndromes such as DRESS (Drug-induced hypersensitivity syndrome/Drug Reaction with Eosinophilia and Systemic Symptoms) [18]. Thus the virus is able to express inflammatory cytokines from both replicating virus and from chromosomally integrated genomes without undergoing productive replication, rendering it only partially responsive to anti-viral therapy such as acyclovir [19]. CIU also responds occasionally to anti-viral therapy, but is often not responsive [8,9].

A role for common herpesviruses in autoimmune disease including CIU based on anecdotal evidence of disease response to anti-viral drugs has been reviewed previously [18]. Elevation of Th2 cytokines could contribute to CIU through increased viral gene and antigen expression in the skin and other tissues leading to observed inflammatory and autoimmune responses. If, in fact, a herpesvirus or -viruses play a role in CIU, it is relevant to ask whether CIU results from a global defect in herpesvirus replication in cutaneous tissues which, for example, appears to be the case in Th2-mediated conditions such as eczema, or rather whether a specific herpesvirus or -viruses are associated with CIU. If evidence exists for a specific herpesvirus or -viruses rather than a global defect, these specific agents associated with CIU would then be candidates for further prospective study in CIU.

Experimental approach and methods to identify candidate herpesvirus pathogens in CIU

As a marker for severe CIU, the author identified a cohort of 10 patients with CIU of more than 1 year in duration who required omalizumab for control of symptoms due to failure of other conventional therapy and who had had previous commercially available viral serology recorded. Patients meeting these criteria were selected randomly by chart review, were not at any risk from this retrospective review of serological data, were not identified or identifiable and had previously signed consent to participate in anonymous clinical research. Review of the serological data was designed to provide preliminary observations for subsequent prospective study.

Patients whose records were analysed further had been on omalizumab for between 1 and at most 6 years. Relapse of CIU had been documented in these patients on discontinuation of therapy, confirming that the underlying disease was still present. An additional patient described anonymously with CIU and autoimmune thyroiditis had

previously signed a written consent for participation in a retrospective chart review and also for 'off-label use' of valacyclovir (Valtrex), as urticaria associated with autoimmune thyroid disease may be a candidate for further prospective placebo-controlled study of both conventional and novel anti-viral therapy [9,20–22]. Because omalizumab therapy was started prior to Food and Drug Administration (FDA) approval of omalizumab for CIU, all omalizumab-treated patients had a co-diagnosis of asthma or wheezing associated with CIU, and some also had autoimmune thyroid disease, as noted, but patients were otherwise healthy. Exact ages are not revealed to adhere to the Health Insurance Portability and Accountability Act of 1996 (HIPPA) guidelines for non-identification, but ranged from early teenage years to late adulthood at the onset of CIU.

Laboratory testing was obtained in all cases from Quest Laboratory (Wallingford, CT, USA) using commercially available FDA-approved tests to permit independent verification of results by other clinicians and clinical researchers. HHV-6 titres were determined using an immunofluorescence dilution assay developed for Quest laboratories by the HHV-6 Foundation (Santa Barbara, CA, USA), in which HHV-6-transformed fibroblasts are incubated with patient serum, fluorescent anti-sera against human IgG, and scored by limiting serial dilutions. Detailed performance characteristics such as receiver operator curves and reproducibility of this HHV-6 assay are not published; however, unpublished data from the HHV-6 foundation suggest that a limiting dilution titre of 1 : 10 or greater is considered positive for past HHV-6 infection, while a 1 : 160 titre or greater may be elevated in healthy unselected blood donors.

Assay enzyme-linked immunosorbent assay (ELISA) titres for HHV-4 were also obtained from Quest for viral capsid IgM and IgG, early antigen IgG and Epstein–Barr virus nuclear antigen (EBNA) IgG, as shown. Assay for HHV-1, HHV-2, HHV-3 (varicella), HHV-5 (cytomegalovirus) and HHV-7, HHV-8 were also obtained from Quest as provided without modification from standard protocols, with results included in the Tables, and did not differ from control values (see text and Discussion). Anti-nuclear and anti-thyroid antibodies were determined by standard Quest assays, as shown. Basophil activation was determined using CD203 fluorescence activated cell sorter (FACS) (assay and positive values shown as determined by the National Jewish Medical and Research Center, Denver, CO, USA).

Experimental findings support a specific role of HHV-6 as a co-factor in CIU rather than a global defect in immunity to herpesviridae

All CIU patients have evidence of HHV-6 infection and most have abnormal and elevated immunoassay titres to HHV-6. Serology data on 10 omalizumab-dependent CIU patients,

Table 1. A cohort of 10 chronic idiopathic/spontaneous urticaria (CIU) patients (numbered 1–10) were all infected previously with human herpesvirus 6 (HHV-6) (denoted H6). Immunoglobulin (Ig)G HHV-6 titres (shown as inverse of limiting dilution value determined by limiting dilution assay, i.e. 80 corresponds to 1 : 80 minimum positive dilution) were elevated compared to control (indicated C) value 10. Years on omalizumab therapy (Y) are shown and did not correlate directly with elevated HHV-6 titres. IgE and IgG levels shown also did not correlate with HHV-6 titres. Serological markers of positive thyroid autoimmunity with serology denoted Y (yes), N (no) or not available (n.a.), anti-thyroglobulin (TG) and thyroid peroxidase (TP) were present in one and three patients, respectively, with patient 3 positive for both markers, but markers of autoimmune thyroid disease also did not correlate with HHV-6 titres.

1	160	5	59	1209	N	N
2	80	6	58	1005	N	N
3	40	4	69	834	Y	Y
4	80	3	16	819	N	N
5	160	1	48	1003	N	Y
6	160	6	324	639	N	N
7	80	6	0	770	N	N
8	360	6	157	1522	N	N
9	10	6	144	1557	N	N
10	160	3	40	973	N	Y
Control	10	n.a.	n.a.	n.a.	N	N
	HHV6	Years	IgE	IgG	TG	TP

denoted patients 1–10, used in this study are shown in Table 1. Other than omalizumab-dependent urticaria, no other preselection or screening of patients was used. Eight of 10 patients were female, and IgE ranged from 0 (undetectable) to approximately 300 IU/ml. These population characteristics are typical of CIU female predominance and lack of immunoglobulin deficiency. No significant differences from reference values were evident in complete blood count (CBC), liver function, cellular immunity (CD4/CD8/CD16/CD19 lymphocytes) or total IgG, IgA, IgM or IgE between patients and reference values (only IgG and IgE data are shown). Serological data obtained after starting omalizumab were analysed for the presence of basophil activation assay (three of 10 positive), anti-nuclear antibodies (ANA) (one of 10 positive) and thyroid autoantibodies (one of 10 positive anti-thyroglobulin and anti-peroxidase, three of 10 positive anti-peroxidase). These results are consistent with previously published data on the prevalence of autoantibodies against basophils (30–50%) and thyroid autoimmune disease (approximately 30 *versus* 5% in healthy controls).

All CIU patients had evidence of previous HHV-6 infection. The statistical significance of this finding in a small sample has not been established (see Discussion). No evidence of positive IgM to herpes 1–7 was present and no patients were positive for IgG against HHV-8 (no commercial assay for HHV-8 IgM is available currently). Remarkably, not only were all CIU patients apparently infected

with HHV-6, but the value of HHV-6 titres was also possibly elevated in CIU patients, as detected in limiting immunofluorescence assay. The HHV-6 assay is considered positive for a reciprocal control value of 10, corresponding to a positive dilution of 1 : 10 serum in healthy adult patients, whereas in CIU the median reciprocal titre was between 80 and 160 in this assay ($P = 0.004$ *versus* control population of 10 patients with normal value 1 : 10). Thus, commercially available serology is consistent with continuing HHV-6 viral gene expression in CIU patients, and it is therefore possible that HHV-6 could be a specific co-factor in CIU.

The youngest patient in the cohort, a teenager at the time of this study (patient 7) developed CIU lasting for more than 5 years after a prolonged apparently viral illness in childhood, progressing to CIU persisting into teenage years. In this study she was found to be positive for HHV-6, with fourfold elevated titres *versus* control (1 : 80 *versus* control 1 : 10). Notably, patient 7 had evidence of response to varicella vaccine (HHV-3 vaccine received in infancy without complications), demonstrating low normal titres for post-HHV-3 vaccine but no evidence of other human herpesvirus infection (Table 2). An adult patient with the lowest HHV-6 titres (1 : 10 titres, patient 9) has been on omalizumab for the longest of all 10 patients with

Table 2. Not all patients were positive for human herpesvirus 4 (HHV-4) patients 7, 8 negative) determined via quantitative measures of viral serology available for Epstein-Barr virus (EBV) (HHV-4) viral capsid antigen (CAP), early antigen (EA) and nuclear antigen (NA), as determined by enzyme-linked immunosorbent assay (ELISA). However HHV-4 ELISA titres to EBV antigens were elevated *versus* control values when present. All patients were also positive for HHV-3 [varicella zoster virus (VZV)] but no patients had ELISA titres above the saturation point of the assay. Elevated titres to HHV-4 CAP and NA of five or greater, indicating greater than saturation value of the ELISA assay, showed a possible correlation with positive basophil activation test [cd203c activation by fluorescence activated cell sorter (FACS) denoted BA], although numbers of patients with positive BA (three of 10) and positive HHV-4 serology (eight of 10 patients) *versus* patients with both elevated EBV and positive BA were too small for statistical analysis. Unfortunately, one CIU patient (patient 5) with highly elevated EBV titres did not have a BA assay obtained [not available (n.a.)].

1	5	0	3.3	N	1.5
2	5	0	1.8	N	1.6
3	4.3	1.2	5	N	1.7
4	4	0	5	Y	2.3
5	5	0	5	n.a.	1.7
6	4.3	1.5	5	N	1.4
7	0	0	0	N	1
8	0	0	0	N	2.7
9	5	0	5	Y	2.1
10	5	4.7	n.a.	Y	n.a.
Control	1.1	1.1	1.1	N	0.9
	CAP	EA	NA	BA	VZV

HHV-6 titres obtained after more than 5 years on therapy. Although, overall, little or no correlation between length of therapy and HHV-6 titres was evident, these observations support a role of HHV-6 as a specific co-factor in CIU, and raise the possibility that HHV-6 titres may decrease during long-term omalizumab therapy (i.e. that omalizumab therapy may have anti-viral effects against HHV-6). Importantly, none of the patients in the omalizumab cohort were treated with valacyclovir or other long-term anti-viral therapy during the study period (see Discussion).

CIU patients have elevated abnormal serological response to HHV-4 (EBV) antigens, but not all CIU patients are HHV-4 infected. After acute HHV-6 infection, acute HHV-4 (EBV) infection is the second most common herpesvirus infection associated with acute urticaria [7]. In serology to herpesviridae, other than HHV-6 obtained from patients in the cohort studied in this work (Table 2), eight of 10 patients with CIU had evidence of past HHV-4 infection and also had elevated ELISA titres to HHV-4 (EBV). HHV-4 IgG titres were particularly elevated to viral capsid and EBNA antigen, which have been proposed as prognostic factors in other autoimmune syndromes such as MS and SLE (see Discussion). For example, five of eight patients with evidence of past HHV-4 infection [positive IgG to VCA, early antigen (EA) or EBNA] had a value greater than the maximum threshold of the ELISA assay, 5 or greater ELISA to HHV-4 viral capsid antigen (VCA) and/or nuclear antigen (EBNA).

Because of the small sample size, and because the absolute value of the ELISA result (greater than assay threshold) was also not known, the statistical significance of this finding is not established. In addition, two of 10 patients had no evidence of past infection with HHV-4, with negative titres to all testing viral antigens. Therefore, HHV-4, but not other common herpesviridae such as HSV-3, might be co-regulated abnormally with HHV-6 in the skin due to increased Th2 cytokine expression in CIU skin, but HHV-4 is not a candidate for an essential or triggering co-factor in all forms of CIU as it is not present in all CIU patients. These observations do not resolve the question of whether, in some cases, CIU might be triggered by HHV-4 independently of HHV-6, or whether HHV-4 alters the phenotype of HHV-6 positive CIU; for example, through increased production of autoreactive IgG.

Further analysis of patient 9, on omalizumab for more than 5 years, demonstrates two additional factors regarding HHV-4 that may be important in the pathogenesis of CIU. First, patient 9 has evidence of autoantibodies against basophils, one of three of 10 patients with these antibodies in the cohort. Patient 9 also demonstrates evidence of extremely elevated titres of IgG to both VCA and EBNA. This serology pattern is strongly suggestive of an abnormal proliferation of an EBV-positive B lymphocyte clone or clones, which can persist for life, as in the syndrome of monoclonal gammopathy of uncertain significance

(MGUS). In support of HHV-4 as a secondary co-factor in autoantibody-positive CIU, a second patient with evidence of autoreactive IgG against basophils in the cohort (patient 10) also has evidence of HHV-4 reactivation with elevated titres to both EBV VCA and EBNA.

Thus it is possible that HHV-6 might trigger the disease process, while involvement of HHV-4, which resides in memory B lymphocytes, could then contribute to an HHV-6 independent chronic form characterized by autoreactive antibodies arising from abnormal EBV-positive memory B lymphocytes. In this putative HHV-6-independent second phase of CIU, autoreactive antibodies against mast cells and basophils produced by activated clonal EBV-positive lymphocytes would maintain the chronic disease state through production of pathogenic autoreactive antibodies arising from 'molecular mimicry', 'epitope spreading' and other immunological mechanisms involving progressive cross-reaction between viral antigens and host antigens [1].

CIU patients do not have an abnormal serological response to HHV-3 (varicella) or other human herpesviruses

As noted in Table 2, all evaluated patients in the cohort had evidence of past infection with HHV-3, an alpha human herpes that is latent in the neural ganglia. However, in contrast to HHV-6 (latent in CD4⁺ helper T lymphocytes) and HHV-4 (latent in CD19⁺ B lymphocytes), CIU was apparently not associated with abnormal response to past infection or vaccination with HHV-3 latent in neurones. All patients except one (patient 7) had been infected with wild-type HHV-3 in the prevaccine era, while patient 7 had received attenuated HHV-3 vaccine. As noted above, patient 7 is of particular interest because, as the youngest patient in the study, this patient had no evidence of any past herpes infection except HHV-6 and HHV-3 attenuated vaccine.

Similarly (data not shown), although four of nine patients had evidence of past infection with HHV-5 (cytomegalovirus), a beta herpesvirus that does not infect lymphocytes, these patients had ELISA titres within the normal range of the positive ELISA assay for past cytomegalovirus infection. Similarly, five of nine evaluated patients had evidence of past infection with an alpha herpesvirus HHV-1. Only one of nine had evidence of past infection with past HHV-2 infection and one of nine had evidence of past HHV-7 infection, and titres were also not significantly different from normal laboratory values for past infection for these herpesviridae within the limits of analysis.

Thus, the abnormal serology evident to past infection with HHV-6 and HHV-4 does not appear to be part of a global immune deficiency (IgG in normal range), atopy (IgE in normal range) or failure in immunity to all herpesviridae, but instead a specific defect related to increased gene expression and possibly reactivation of HHV-6 and

Table 3. An omalizumab-naïve patient with chronic idiopathic/spontaneous urticaria (CIU) seen first in May 2006 demonstrated evidence of active autoimmune thyroiditis, including highly elevated thyroid stimulating hormone (TSH) (patient TSH 76 *versus* laboratory standard less than 5), and an extremely elevated enzyme-linked immunosorbent assay (ELISA) titre of antibodies to thyroglobulin (TY) and thyroid peroxidase (TP). Her CIU was controlled by high doses of non-sedating anti-histamines, oral corticosteroids and compliance with thyroid hormone replacement therapy, but she continued to have flares of CIU requiring oral corticosteroids until 2013. In January 2013 she started a trial of anti-viral therapy with valacyclovir (valtrex) 500 mg twice daily for possible HHV-6 reactivation contributing to CIU and thyroiditis based on stable and extreme elevation of human herpesvirus 6 (HHV-6) titres (640 reciprocal titre in 2008 and 2011). At follow-up in July 2013 she reported better control of both CIU and thyroid disease with evidence of normalized TSH. In January 2014 she had discontinued anti-viral therapy for 6 months and showed some evidence of thyroiditis (TSH again elevated) and anti-viral therapy was restarted. Decreased HHV-6 activation correlated with improvement in clinical CIU first evident in 2014 has continued on anti-viral therapy until the present, with HHV-6 inverse titres reduced fourfold on therapy from 640 to 320 and subsequently to 160 in 2015. Serology did not change for other herpesviruses by ELISA, including Epstein–Barr virus (EBV) [denoted capsid antigen (CAP), early antigen (EA) and nuclear antigen (NA), varicella zoster virus (VZV), as in Table 2].

	TSH	TP	TG	CAP	EA	NA	HHV-6	VZV
ptxmay07	70.6	1000	3000	5	2.3	5		
ptxnov08	17.6			5	2.1	5	640	2.2
ptxjul11				5	2.1	5	640	2.4
ptxjul13ac	4.1	287	3000					
ptxjan14	11.3	236	3000	5	2.3	5	320	2.1
ptxaug14ac	5							
ptxjan15ac	5						160	

HHV-4, presumably in both lymphocytes and epithelial tissues. Notably, HHV-4 and HHV-6 both establish latency in lymphocytes (CD19 memory B lymphocytes and CD4 helper T lymphocytes, respectively), and thus might be particularly sensitive to and associated with cytokine-mediated inflammation in cutaneous tissues evident in CIU.

Abnormal serology to HHV-6 is not an artefact related to omalizumab

In addition to the cohort of patients with CIU on omalizumab described above, charts of omalizumab-naïve patients were also reviewed retrospectively for evidence of an association between elevated HHV-6 immune response and autoimmune thyroiditis, a condition associated with increased replication of HHV-6 in thyroid tissues [12]. This analysis was to address the possibility that the elevated serology to HHV-6 (and HHV-4) noted in this cohort of CIU patients on omalizumab was an artefact related to omalizumab rather than an underlying feature of the disorder.

Serological data from a representative CIU patient not treated with omalizumab are shown in Table 3. This

patient, with both CIU and autoimmune thyroid disease, was non-compliant with thyroid hormone therapy at initial evaluation, as she maintained that taking thyroid medications contributed to exacerbations of her urticaria. As shown in Table 3, initial highly elevated titres to HHV-6 (1 : 640) decreased to 1 : 320 and then to 1 : 160 after anti-viral therapy with valacyclovir, while no other changes in viral serology were noted. Associated with anti-viral therapy, she also noted improved control of CIU, which had been poorly responsive to antihistamines and controlled only with oral corticosteroids. Her CIU has remained in remission off medications for the past 3 years on anti-viral therapy at standard and non-toxic dosing, with the exception of a flare of CIU and thyroiditis associated with a period when she discontinued anti-viral therapy for 6 months. After restarting acyclovir therapy, serological titres to HHV-6 have decreased further, along with thyroid peroxidase autoantibody titres correlating with remission in both CIU and thyroid-related symptoms.

Remarkably, improvement in CIU correlated well with decreased thyroid-stimulating hormone (TSH) levels, and also with decreased titres to HHV-6 but not HHV-4. These observations suggest that abnormal serology to HHV-6 and HHV-4 when present are not an artefact related to use of omalizumab, but instead are associated with the underlying inflammatory pathology. A plausible interpretation of this patient and similar patients with concurrent CIU and thyroid disease is that both omalizumab and anti-viral therapy may be most effective if the CIU is associated with evidence of HHV-6 active replication, such as acute thyroiditis and elevated titres to HHV-6. Conversely, patients with long-standing CIU associated particularly with autoantibodies and abnormal response to HHV-4 are not found to respond to anti-viral therapy, but still may respond to omalizumab.

A novel paradigm of CIU pathogenesis as 'chronic viral urticaria' (CVU) similar to DRESS

Many studies of CIU have focused on the symptoms of CIU and associated phenomena such as autoantibodies [1]. Based on analysis of omalizumab-dependent CIU patients, a working model or paradigm of CIU pathogenesis is presented as a syndrome related to partial or complete reactivation of a herpesvirus or viruses in cutaneous tissues, most consistent with serological data from the cohort of patients summarized in Table 1. Because of the small sample size and retrospective nature of this study, additional studies will be required as shown to confirm or refute any model presented based on these observations. In this model (Fig. 1), the initial triggering event in CIU is localized cutaneous inflammation due to reactivation of HHV-6 infected CD4 T lymphocytes following primary infection with HHV-6 occurring in early childhood. In support of this model, recent evidence confirms that a viral-related

Chronic Viral Urticaria (CVU) Paradigm:

A) Latent and Lytic HHV-6 Infection

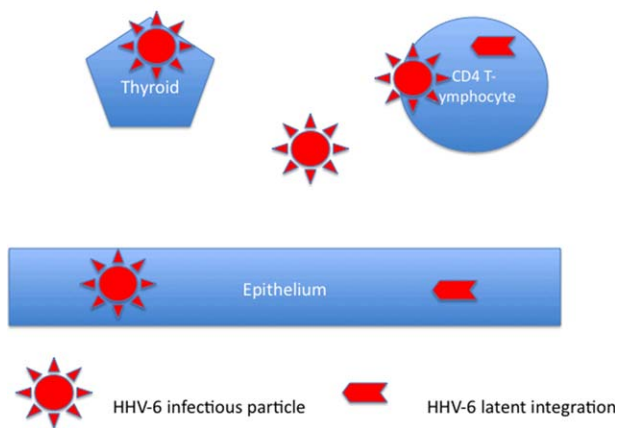


Fig. 1. A model of human herpesvirus 6 (HHV-6) reactivation in chronic idiopathic/spontaneous urticaria (CIU): HHV-6 shown in red exists in latent form in CD4 T lymphocytes. In latency the virus is integrated into the telomeres of host chromosomes, depicted as a linear chromosomal element. In latency the virus may still express gene products (virokines, micro-RNA) in the absence of viral or host DNA replication, but the virus is not sensitive to anti-viral agents such as acyclovir/valacyclovir that inhibit viral DNA replication. Following viral reactivation in response to cellular inflammatory signals that are not well characterized the virus forms infectious particles depicted as spheres, and this stage of the viral life-cycle is blocked by valacyclovir anti-viral therapy that blocks a viral DNA polymerase. Infectious viral particles also undergo lytic replication cycles in the thymus and epithelial tissues.

Th2-like pathology is evident in the skin during HHV-6-associated drug allergy syndromes such as DRESS with or without evidence of active viral replication [23].

Omalizumab, a monoclonal antibody against the IgE receptor binding site of IgE, is a highly effective therapy for the symptoms of CIU but does not seem to affect the underlying pathology of the disease, as symptoms return soon after the medication is discontinued. In the model shown in Fig. 2, CVU related to HHV-6-dependent inflammation would respond to omalizumab through the anti-inflammatory effects of IgE receptor blockage on downstream Th2 cytokines such as interleukin (IL)-4 and IL-13, as well as what effects depletion of surface IgE receptor might have on autoreactive antibodies when present. Previous models of CIU pathogenesis that are centred on autoreactive antibodies can explain some of the features of the disease, but cannot explain the apparent lack of these antibodies in a significant population of patients and the absence of changes in autoreactive immunoglobulin in patients who respond none the less to omalizumab therapy.

Perhaps many years after primary HHV-6 infection, reflecting altered Th2 cytokine balance in the host due to hygiene, other illness or factors reactivation of gene expression from HHV-6-infected CD4 lymphocytes and epithelial

cells could trigger a chronic inflammatory state associated with clinically observed autoimmune pathology as well as depletion of mast cells in the skin and basophils in the serum. This inflammatory state would be sufficient to trigger urticaria analogous to the exanthems of primary HHV-6 and also urticarial syndromes typical of primary infection with other herpes pathogens or reactivation in DRESS. Long-term cutaneous inflammation would also stimulate proliferation *in situ* of local autoreactive HHV-4 positive B lymphocyte clones. Because of the small size of patients reported in this study, many questions remain unresolved; for example, whether long-term use of omalizumab is correlated with decreased viral reactivation of HHV-6 (as suggested by patient 9). Related questions include whether the level of elevation of initial HHV-6 and HHV-4 titres predict a clinical response to omalizumab and whether decreased HHV-6 serology correlates with a clinical response to therapy.

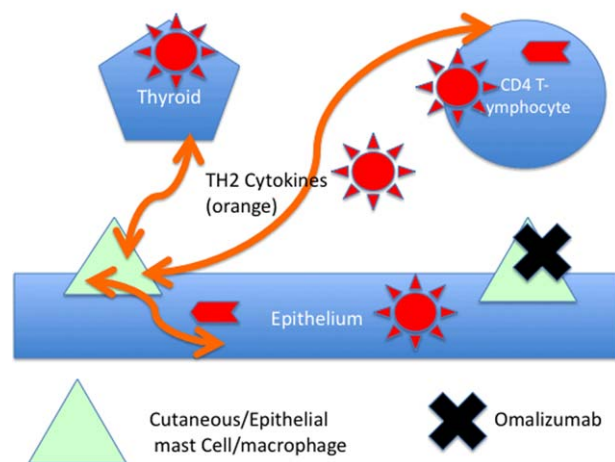
Chronic Viral Urticaria (CVU) Paradigm:
B) TH2 cytokines and viral load, omalizumab

Fig. 2. A model of the role of omalizumab therapy in chronic idiopathic/spontaneous urticaria (CIU) through decreased human herpesvirus-6 (HHV-6) gene expression: in this model omalizumab might interfere with immunoglobulin (Ig)E-sensitive T helper type 2 (Th2)-driven mast cell and basophil-mediated inflammation and thus decrease viral gene expression and viral mediated inflammation. Epithelial immune cells such as mast cells and macrophages may respond to viral infection with increased inflammatory cytokine and chemokine production, as suggested by recent experiments using a transgenic CD46-positive murine model of neuroepithelial HHV-6 infection. In the neuroepithelium murine model, production of host cytokines and chemokines does not require viral replication or production, and thus would not be sensitive to anti-viral therapy. An important prediction of this model is that omalizumab might be of use in some severe drug allergy syndromes such as drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DRESS), also apparently related to both mast cell activation Th2 cytokines and increased reactivation of both HHV-6 and HHV-4.

An important and long-standing observation in CIU is an underlying inflammatory process that, by analogy to DRESS, could be driven by increased gene expression of a chronic viral pathogen. A common underlying aetiology for the underlying inflammation in both DRESS and CIU could open a new window into definitive or curative therapies both in CIU and DRESS [23]. Long-standing clinical observations in CIU have suggested that patients who report multiple drug and antibiotics allergy often have underlying CIU. Another clinical question concerns whether the post-viral model proposed is applicable to all forms of chronic urticaria, including physical urticarial syndromes, which also respond to omalizumab. If, in both cases, the underlying defect is related to abnormal gene expression of HHV-6 in cutaneous tissues, then monitoring of HHV-6 or related markers for viral infection could be useful in predicting the response to anti-viral therapy. Similarly, mRNA or other gene-profiling methods might be helpful in discriminating between patients with active HHV-6 cutaneous replication and those with inflammation not related to active HHV-6 replication, but perhaps post-viral expression of inflammatory signals from stably integrated viral genomes or autoimmune response.

Potential relevance to other cutaneous and neuroepithelial autoimmune syndromes

This retrospective study was modelled in part on a large and increasing literature regarding the role of common viral pathogens in common autoimmune diseases. Autoimmune diseases such as MS and SLE are, like CIU, apparently increasing in prevalence in modern societies due to factors such as hygiene and altered composition of the human microbiome and virome [9,16]. For example, extensive observations suggest that MS, an autoimmune disease affecting the neuroepithelium, and SLE, associated with epithelial disease, are associated with abnormal response to chronic infection with some herpesviridae, specifically HHV-4 and HHV-6 [9,16]. Autoantibodies are often or always present in these diseases, and HHV-4-specific IgG antibodies to viral proteins, such as VCA or EBNA, correlate with the underlying pathology which seems to be related to increased herpesvirus reactivation and gene expression. HHV-4 also encodes a copy of the immunosuppressive cytokine IL-10 termed a virokin that may alter local immune tolerance pathways, and most chronic viral pathogens locally target suppression of autoimmunity by IL-10 [24].

In other autoimmune syndromes such as MS and SLE, serology to HHV-4 antigens is detected years or decades before presentation of clinical disease, and serology to HHV-6 antigens may correlate with disease progression and exacerbations. The data presented in this study suggest that only HHV-6, rather than HHV-4, is present in all cases of CIU; however, a common mechanism or mechanisms may underlie autoimmune pathogenesis, because each

virus is capable of triggering both systemic and localized inflammation through a variety of mechanisms including viral-encoded cytokines (virokines), and viral-encoded micro-RNA. One intriguing possibility is that HHV-6 and HHV-4 viruses are associated particularly with autoimmune syndromes, as they replicate in lymphocytes and can activate endogenous superantigens expressed on lymphocytes encoded by extinct retroviruses or human endogenous retroviruses (HERV) [9].

CIU thus could provide a useful means to study human autoimmune disease *in situ* in humans, as a skin biopsy can be obtained in CIU much more readily than a brain biopsy in MS or other systemic autoimmune syndromes. CIU, a common clinical syndrome, also does not have the costs or experimental uncertainty of extrapolation from animal models to human disease in evaluation of response to therapy. As in scleroderma, CIU seems to be an autoimmune and inflammatory syndrome targeting the skin, and thus viral gene expression in host tissues may similarly alter local innate immune responses [11]. Herpesvirus replication and gene expression are not independent of the host, but instead are influenced by host Th2 cytokine and other inflammatory cytokine expression, and viral gene expression in turn interacts with host Th2 cytokine expression in epithelial tissues [18]. As the prevalence of both Th2-mediated atopic disease and autoimmune disease increases in modern societies it is probable that increased Th2 bias in the skin would also predict an increased prevalence of CIU.

Discussion

To paraphrase Sherlock Holmes, the creation of a physician, Sir Arthur Conan Doyle, the clinical investigator must consider all the possibilities, discard those that do not fit the facts of the case and whatever remains, however improbable, is the solution to the mystery. CIU is a mysterious collection of overlapping syndromes which seem to be increasing in prevalence, often requiring prolonged therapy [1,25–27]. HHV-6 is ubiquitous in human populations, possibly having arrived from infection in the distant past from other simian hosts, and its role in chronic human diseases may be under-estimated [28].

In this report, commercially available serology was used to analyse a population of patients with omalizumab-dependent CIU on therapy for between 1 and 6 years to determine whether HHV-6 or other human herpesviruses could play a role as co-factors in CIU. Results of serology in a cohort of patients with omalizumab-dependent CIU demonstrated that all patients with omalizumab-dependent CIU had evidence of past infection with HHV-6 and serology, suggesting an increased host immune response. A limitation of these studies is the lack of a standardized assay for HHV-6 or published performance data of the HHV-6 serological assay.

Previous published and unpublished serological studies using this and related serological assays have suggested that

more than 90% of the US adult population is HHV-6 infected, with approximately 1% of the population bearing a germline copy of the virus [13,29–32]. Alternatively, newer molecular characterizations of the entire human virome suggest that more than 90% of adults have evidence of infection with HHV-4, while only approximately 70% with HHV-6 [33]. In part, these differences are related to the lack of current serological assays to discriminate between two distinct strains of HHV-6 (termed HHV-6A more common in HIV and African patients) and HHV-6B (more common in other populations), as well as serological cross-reaction between HHV-6 and other related human herpesviruses. Thus the hypothesis suggested in this work, that HHV-6 mediated inflammation contributes to CVU, should be tested either in a prospective serological study of a larger sample of CIU patients *versus* age-matched controls and also by cytological or nucleic acid-based expression analysis of localized viral gene expression in CIU-mediated cutaneous tissues with or without evidence of increased systemic immune response to HHV-6.

Importantly, while serological evidence of a highly elevated response to HHV-6 may predict the response to anti-viral therapy for CIU, as discussed in this report, in a single patient with autoimmune thyroid disease, routine treatment with anti-viral therapy of all CIU patients is currently not advisable, because many CIU patients may have only localized cutaneous viral gene expression or post-viral inflammation similar to DRESS in the absence of viral replication targeted by current anti-viral agents [23]. As in DRESS, specific indications for anti-viral therapy in CIU remain to be determined. In addition, as some of the benefits of omalizumab in CIU may be due to cytokine-mediated effects on underlying viral gene expression, in the future omalizumab might also be evaluated as a novel form of anti-viral therapy with proven clinical efficacy and safety in CIU and possible efficacy in HHV-6 related syndromes such as DRESS.

In this report, an attempt has been made to correlate CIU pathology with serological evidence of reactivation of latent herpesviruses using a population of severe CIU patients dependent on omalizumab. Analysis of this population suggested that HHV-6 reactivation and gene expression, possibly co-regulated with bystander effects on HHV-4, could play a role in CIU pathogenesis. These observations have implications both for the pathogenesis and therapy of CIU, drug allergies such as DRESS and also other autoimmune diseases, particularly autoimmune thyroid disease long associated clinically with CIU.

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Disclosure

None.

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