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INVESTIGATIVE REPORT

Investigation of Cytomegalovirus and Human Herpes Viruses 6 and 7 as Possible Causative Antigens in Psoriasis

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Psoriasis is probably a T-cell-mediated autoimmune disease. Infectious models of autoimmune diseases have been proposed and in psoriasis, it has been suggested that there may be molecular mimicry between streptococcal antigens and epidermal keratins. The immunological profile of stable psoriasis plaques suggests, however, that viral antigens may be important. We investigated, using polymerase chain reaction techniques, whether DNA from either cytomegalovirus (CMV) or human herpes viruses (HHV) 6 and 7 is present in the skin of patients (n= 10) with chronic plaque psoriasis. We also investigated 29 patients for the presence of serum IgG to CMV. We found no evidence of CMV or HHV 7 DNA in psoriasis plaques although DNA for HHV 6 was detected in both involved and uninvolved skin in 1 out of 10 patients. There was no statistically significant increase in prior CMV infection, as assessed by the presence or absence of serum IgG to CMV, in psoriasis, compared to our local population. Although there is circumstantial evidence that viral antigens may be important in the pathogenesis of psoriasis we found no evidence to link infection with CMV or HHV 6 and 7 with subsequent development of chronic plaque psoriasis. *Key words: Th1 cytokines; Vβ restriction; polymerase chain reaction.*

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Although the ultimate pathogenesis of psoriasis remains unclear, there is strong evidence suggesting it to be a T-cell-mediated autoimmune disease (1). Immunohistochemically, psoriatic plaques are characterized by a predominantly dermal infiltration of CD4+ T cells and intraepidermal CD8+ T cells (1, 2). These T cells produce multiple cytokines, mainly in a Th1 (interferon-ψ and interleukin-2) profile (3). Classically, the stimulation of CD8+ T cells with production of Th1 cytokines occurs in response to intracellular bacterial and viral antigens (4). Recently it has been shown that there is clonal expansion of certain subsets of CD8+ T cells within psoriatic plaques (2). These T cells appear to respond in an antigen-specific manner (5). Viral antigens have been proposed as important in the immunopathogenesis of both type 1 diabetes mellitus (6) and multiple sclerosis (7, 8). These diseases share similar immunological features to those of psoriasis, which include: (i) Vβ restriction of T-cell receptors; (ii) a T-cell predominant inflammatory infiltrate in the target organ; (iii) a local excess of Th1 cytokines; and (iv) development of the disease is dependent on interactions between a genetic predisposition and environmental factors (6–8).

Any virus proposed as a candidate aetiologic agent for psoriasis would need to (i) be common; (ii) have a predominantly asymptomatic primary infection; (iii) possess the ability to remain latent and to reactivate within the host; (iv) be capable of being detected in skin; and (v) be able to induce a local inflammatory response. Human herpes viruses (HHV) 6 and 7 and cytomegalovirus (CMV) commonly produce chronic, asymptomatic infections and may cause local immune-mediated reactions (9, 10). CMV can stimulate local production of tumour necrosis factor-α and interleukin-2 (11), both of which are increased in psoriatic plaques (1, 3). Infection with HHV 6 and 7 is ubiquitous and the majority of infections are asymptomatic (9, 10). Furthermore, HHV 6 has been implicated in the pathogenesis of roseola and multiple sclerosis (9).

We hypothesize that the psoriatic phenotype may occur as the result of an immune reaction to normally asymptomatic local viral, or similar, infection in genetically predisposed individuals. We therefore investigated whether patients with chronic plaque psoriasis exhibit evidence of infection with CMV and HHV 6 and 7.

MATERIAL AND METHODS

Patients aged ≥18 years with chronic plaque psoriasis were recruited from our psoriasis clinic. After written informed consent (approved by the Salford and Trafford Local Research Ethics Committee) blood was taken from 29 patients. Ten other patients had 4 mm diameter punch skin biopsies (using lignocaine and adrenaline as local anaesthetic) taken from involved and uninvolved skin. None of the patients were on systemic therapy or receiving phototherapy for psoriasis. Serum antibodies to CMV (IgG) were measured by ELISA. Infection with HHV 6 and 7 is ubiquitous by adulthood and therefore serological tests for antibodies to these organisms were not performed. Biopsies from involved and uninvolved skin were examined for DNA of CMV, HHV 6 and 7.

Skin biopsies were initially snap-frozen in liquid nitrogen and stored at – 80 C. The biopsies were disrupted during thawing by using a glass homogenizer. DNA was extracted from this material with guanidine thiocyanate and isopropanol precipitation. The sequences of the human CMV primers were 5′-TGCAGTTTGGTCCCT-TAAAG-3′(233C) and 5′AAGAATC CTCA CCTGG CTTA-3′ (724C) (12), which were derived from the DNA sequence of the CMV phosphoprotein gene and gave a product size of 171 bp. The HHV 6 primer sequences were 5′AAGCTTGCACAATGCCAAAAA-ACAG-3′ (H6-6) (13) and 5′CTCGAGTATGCCGAGACCCC-TAATC-3′ (H6-7) and gave a product size of 223 bp. HHV 7 primers were 5′CAGAAATGATAGACA GATGTTGG-3′ (HV-10) and 5′-TAGATTTTTTGAAAAAGATTTAATAAC-3′ (HV-11) (14), with a product size of 123 bp. The β-globin primers were 5′-

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GAAGAGCCAAGGACAGGTAC-3′ (GH20) and 5′CAACTT-CATCCACGTTC ACC-3′ (PC04) (15), with a product size of 268 bp.

The PCR mixture comprised 10 mM Tris/HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM each deoxynucleoside triphosphate, 0.2 μM each of the appropriate primers, 1.25 U of Amplitaq Gold polymerase (Perkin-Elmer, Akron, OH) and 5 μl of appropriate DNA sample to a final volume of 50 μl. Every PCR run included a contamination control, where sterilized, distilled water replaced the DNA sample, and an extraction control, where sterilized, distilled water was extracted alongside the specimens and added to a PCR mix. Each reaction mixture was overlaid with one drop of mineral oil to prevent evaporation. An initial denaturing step at 94 C for 9.9 min was followed by 40 cycles at 94 C (1 min), 55 C (1 min) and 72 C (1 min) on a PHC-1 thermal cycler (Techne, Cambridge, UK). The amplification products were analysed by ethidium bromide staining after electrophoresis in 6% polyacrylamide gels and the anti-contamination measures were as described previously (16). These included separate rooms for preparation of the reaction mixtures and for preparation and addition of DNA extracts and a third room for product analysis. Plugged pipette tips were used throughout.

Positive control DNA comprised: (a) human genomic DNA (Boehringer, Roche Diagnostics, UK); (b) the *Hind*III restriction fragment J of HCMV (AD169) DNA cloned into plasmid pAT153 (15); and (c) the *Hind*III restriction fragment pHD5X of HHV 6 DNA (17) and HHV 7-infected cell DNA (Abgene, Epsom, UK). Using these control DNAs, the β-globin and HHV 6 PCRs had sensitivities of ω 10 copies per reaction mixture, the CMV PCR could detect 10–100 copies and the HHV 7 PCR could detect a $10^{-6.5}$ dilution of HHV 7-infected cell DNA. For HHV 6-positive samples, 5 μl of reaction product was digested overnight with 5 units of *Ava*II (Boehringer) according to the manufacturer's instructions (17). The products were analysed by polyacrylamide gel electrophoresis.

RESULTS

Twenty-one of 29 (72%) patients had IgG antibodies to CMV, compared with 50% in our general control population (18). Antenatal screening has suggested that the incidence of CMV infection increases by 1% per year after adolescence (19). As the mean age of our population was 44.6 ± 10.8 years (range 23–69 years), there was thus no significant increase in CMV infection in our patients with psoriasis. There was no correlation between infection with CMV, clinical severity of psoriasis and previous treatment with systemic therapy. CMV and HHV 7 DNA were not detected in either involved or uninvolved skin in any patient. DNA for HHV 6 subgroup A was detected in both the involved and uninvolved skin in 1 of the 10 patients.

DISCUSSION

Infectious models for the pathogenesis of autoimmune diseases have been proposed previously and several studies of diabetes (6) and multiple sclerosis (7, 8) have supported this hypothesis. Such an infectious model has been proposed for psoriasis in relation to streptococcal infection, with possible molecular mimicry between the streptococcal M-peptide and keratins (20). While streptococcal/keratin mimicry may play a role in the development of psoriatic plaques, the immunological characteristics of such plaques suggest that other factors may be important. These immunological characteristics include: (i) Vβ restriction of T cells within psoriatic plaques; (ii) a predominantly Th1 cytokine profile; and (iii) activated CD8+ T cells. All of these factors are indicative of an immunological response to the presence of local stimulating viral or intracellular bacterial antigens (4, 5). There is circumstantial evidence to support this concept, which includes the observations that zidovudine and hydroxyurea, agents with anti-retroviral activity (21), are effective in treating psoriasis. Furthermore, there is evidence that DNA of human immunodeficiency virus (22), hepatitis C virus (23) and human papillomavirus (24) is present in psoriatic plaques.

We investigated whether CMV or HHV 6 and 7 are possible causative or contributory antigens in psoriasis. We chose these viruses because infection with them is common and because HHV 6 has been proposed as a possible antigen in multiple sclerosis, which shares several immunological characteristics with psoriasis. HHV 7 and CMV are similar viruses to HHV 6 and can also evoke immunological reactions (11). Although a recent study (25) found evidence of increased CMV antigenemia in psoriasis, there was no evidence of CMV antigens in the skin. As CD8+ T cells in psoriasis appear to act in an antigen-specific manner, the absence of CMV antigens in the skin would suggest that CMV infection is not a primary event in either the development or maintenance of psoriasis plaques. The presence of HHV 6 DNA in the skin of only 1 of our patients suggests that this organism is unlikely to be of significance. It has been suggested that multiple sclerosis is a manifestation of several disease processes (8). Similarly, psoriasis may be considered as a final cutaneous manifestation of several pathomechanistic processes. These processes could include persistent viral infection of the skin resulting in skin homing of specific CD8+ T cells and subsequent release of cytokines, growth factors and angiogenic factors by both activated T lymphocytes and keratinocytes. Potential viral candidates as antigens for psoriasis would have to be common, capable of causing an immunological reaction and present in skin. Future studies will concentrate on searching for likely viral candidates for psoriasis.

In conclusion, there is circumstantial evidence that local viral or intracellular bacteria may be responsible for the immunological events seen in chronic plaque psoriasis. However, we have shown that infection with CMV and HHV 6 and 7 is unlikely to play an important role in psoriasis pathogenesis. Candidate infectious agents which may be important to investigate include retroviruses, Coxsackie viruses and Epstein–Barr virus.

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