occurred in the pedigree. The eyebrows and eyelashes in all these patients were normal. Apart from the noted congenital alopecia of the scalp, there were no associated abnormalities of the skin, nails, or teeth. Sweating, heat tolerance, growth, and development were all normal. Skin biopsy obtained from the scalp of the 2-year-old boy (IV 8) revealed a decreased number of atrophic hair follicles, but the eccrine glands were normally distributed. Results of routine laboratory tests, including sex hormones and aminoacid values were all normal. Other defined syndromes that might have been associated with alopecia were ruled out.

Although Sobajima[3] reported a pedigree with isolated congenital alopecia in 1927, in which X-linked recessive inheritance could not be ruled out, others[4] considered that this pedigree could have been classified as ectodermal dysplasia. Thus the family described here is the first pedigree with isolated congenital alopecia in which X-linked recessive inheritance has been confirmed.

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The painful eye

SIR—In a case report by MacConnell and Ferro (Nov 11, p 1269)[1] the problem of distinguishing between infection and inflammation in the eye was highlighted. Although acute anterior uveitis (AAU), an endogenous inflammatory disorder, typically presents with the rapid onset of a painful red eye, the diagnosis of metastatic endophthalmitis as the cause of acute intraocular inflammation must always be considered and excluded in patients who are unwell, pyrexial, have an acutely inflamed joint or septic focus elsewhere.[2] Clinical pointers towards infection include rapidity of onset, severe pain, eyelid swelling, hypopyon, vitreous opacification, and the presence of a relative afferent pupillary defect. None of these signs, however, are pathognomonic of infection, and a high index of clinical suspicion is crucial in establishing the diagnosis.

If there is any clinical doubt, aqueous and vitreous sampling must be undertaken quickly and intravitreal antibiotics given—most commonly a combination of vancomycin (1–2 mg) with amikacin (400 μg) or ceftazidime (2·25 mg).[3] The outcome of endophthalmitis depends upon both the virulence of the infecting organism and the rapidity in initiating appropriate antibiotic therapy. Delaying therapy while awaiting culture results from intraocular samples, which are frequently negative, usually means that treatment will be started too late.

Infecting organisms enter the eye predominantly via one of two routes: directly through a penetrating wound of either surgical or traumatic origin, or from the blood from a distant focus of infection—eg, lung abscess, septic joint, or intravenous catheter. Topical antibiotics alone are of little value in the management of endophthalmitis, and the majority of systemic antibiotics do not adequately cross the intact blood-retinal barrier, with a few notable exceptions such as the quinolones. For this reason systemically administered ciprofloxacin is often used in the management of endophthalmitis. The integrity of the blood-retinal barrier is impaired to some extent in inflamed eyes, allowing antibiotics to penetrate the eye but without achieving adequate therapeutic levels. In addition, the acute inflammatory response engendered by severe intraocular infection compromises visual function, and intraocular or systemic steroids are frequently employed to ameliorate this.

Exogenous or endogenous infection must always be considered in any acutely inflamed eye, and if this diagnosis cannot be excluded on clinical grounds, the eye should be treated as infected to ensure that valuable time is not lost. The eye is rarely the source of metastatic infection; much more commonly it is the target. As the case report clearly illustrates, a hot eye may be the first sign of an impending clinical catastrophe.

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HHV8 and skin cancers in immunosuppressed patients

SIR—A new human herpesvirus (HHV8) was first discovered in AIDS-associated Kaposi's sarcoma (KS) lesions[1] and subsequently in all forms of KS, including that of HIV-negative immunosuppressed transplant recipients.[2] Rady and colleagues[3] reported that HHV8 is present in various skin tumours in immunosuppressed transplant patients and conclude that HHV8 is more widely spread and is possibly activated in skin lesions (including KS) during immunosuppression. We have investigated the presence of HHV8 sequences in 37 skin squamous carcinoma biopsy specimens from 25 immunosuppressed and six immunocompetent individuals. In addition, we tested skin biopsy specimens from nine bone-marrow transplant recipients with graft versus host (GVH) disease. The quality of DNA extracted from paraffin-wax-embedded tissue was tested by amplifying for an endogenous retrovirus (erv3), present at two copies per diploid genome.[2]

We used previously published nested primers (amplifying a 233 basepair [bp] product derived from ORF 26) to test for the presence of HHV8.[2] Initially we found that 14/37 (38%) of squamous tumours were positive. After cloning, we sequenced all positive cases, and found all sequences identical, suggesting contamination. We then retested all cases with non-overlapping primers derived from the conserved HHV8 major capsid protein gene, ORF 25 (outer primers 5'-AGGCAACGTCAGATGTGAC-3', 5'-GAAATTACCCACGAGATCGA-3; inner primers 5'-CATGGGAGTACATTGTCAGGACCTC-3', 5'-GGAAT-TATCTCGCAGGTTGCC-3).[4] All cases, including GVH disease, were negative with this set of primers.

DNA was re-extracted from all specimens, amplified with both sets of primers, and all samples were again negative. Sensitivity of our PCR for the new set of HHV8 primers was tested by amplifying serially diluted genomic DNA from an HHV8 containing cell line (HBL-6). This cell line is derived

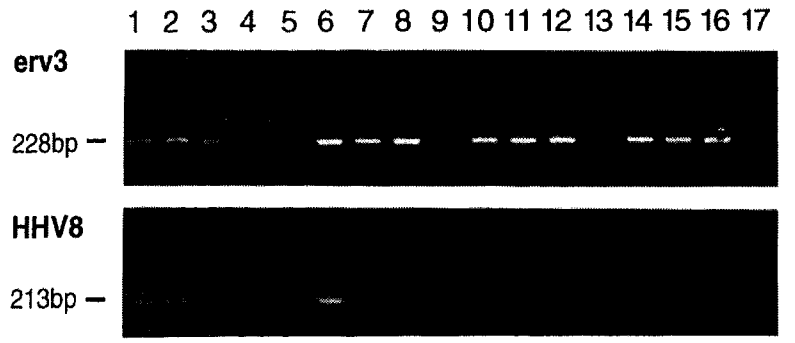

Figure: **Nested PCR amplification of erv3 (two copies/diploid genome) and HHV8 (ORF 25)**
Lanes 1–5, ten-fold dilutions (10^{-3}–10^{-7}) of DNA from HHV8 containing cell line (HBL-6); lanes 6–9, 10^{-2}–10^{-5} dilutions of DNA extracted from a paraffin-wax-embedded KS biopsy specimen; lanes 10–13 and 14–17, undiluted and 10^{-2}, 10^{-4}, 10^{-5} dilutions of DNA extracted from two paraffin-wax-embedded squamous carcinomas. PCR bands visible above nested amplification products caused by residual primers from first-round amplification.

from the same body-cavity-based lymphoma as the BC-1 line (estimated 50 copies HHV8 per cell).[5] The nested PCR for HHV8 detects viral DNA at a ten-fold lower dilution than does amplification of a single copy gene (erv3), indicating between ten and 100 HHV8 copies per cell (figure). In paraffin-embedded KS tissue the endpoint dilution for HHV8 and erv3 is identical, suggesting that between 10% and 100% of cells carry the equivalent of one virus genome. We find that even at the levels of PCR sensitivity indicated (detection of 1 copy HHV8 per 10 000 uninfected cells), we still fail to detect the presence of HHV8 in squamous carcinomas (figure).

We therefore cannot confirm Rady and colleagues'[3] finding of HHV8 in squamous carcinomas from immunosuppressed patients. Furthermore, six squamous carcinomas from immunocompetent patients and nine biopsy specimens from bone-marrow transplant patients with GVH disease were also negative. Our results highlight the difficulties of contamination during PCR amplification and the importance of the use of non-overlapping primer sets and sequencing to confirm positive results, as well as the need to devise PCR sensitivity. Our results argue against the suggestion that HHV8 is a widespread virus reactivated by immunosuppression in these skin tumours. HHV8 sequences have been detected in greater than 95% of nearly 200 KS biopsy specimens in various studies, supporting a role for this agent in the pathogenesis of KS.[1,2,4]

We thank Ken Welsh for clinical information. This work was supported by CRC, Mercury Phoenix Trust, and the MRC.

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Authors' reply

SIR—In our report documenting the presence of a new herpesvirus (HHV8) in various skin tumours from four immunocompromised organ transplant patients, all appropriate positive and negative controls were used. Direct sequencing of the 233 bp fragment in our positive samples demonstrated variants, thus futher excluding contamination. Therefore, our results confirm that HHV8 is more widespread than originally described.

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1 Rady PL, Yen A, Rollerson JL, et al. Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. *Lancet* 1995; **345:** 1339–40.

Endocrine hypothesis for declining sperm count and rising incidence of cancer

SIR—There is a need to re-examine the hypothesis[1] that it is the endocrine effect of environmental chemical pollution that is the most significant cause of the apparent declining sperm count and rising incidence of testis cancer in man.

In 1990 it was postulated that gonadal atrophy, because it leads to increased gonadotropin drive, thus allowing less time for DNA repair, provides the link between declining sperm counts and rising incidence of testis cancer.[2] If the view that the endocrine effects of these chemicals is the dominant cause of infertility one might expect the follicle stimulating hormone (FSH) values to be low. In fact high serum FSH at diagnosis of testis cancer is a risk factor for developing a second testis cancer.[3] As germ cells are the most sensitive to genotoxic agents, we suspect that the genotoxic, rather than the sex hormone mimicking, effects of chemical pollution may be at least co-dominant, producing their effect before fertilisation rather than in utero. As some of these chemicals are also immunosuppressive, persistence of infectious agents could also be playing a role in gonadal atrophy and testis cancer. It is already known that viruses such as mumps can induce gonadal atrophy[2] and there is evidence that sexually acquired infection is a risk factor for testis cancer.[2]

Supporting evidence for the view that persistent non-specific bacterial and viral infection could be a more frequent factor in testis cancer than previously appreciated comes from a detailed analysis of a small unselected cohort of follow-up patients treated by this unit and undergoing screening for risk factors. More than half of these patients had elevated FHS values as evidence for damage to the remaining testis and one third had fluctuating episodes of minor testicular pain and tenderness suggesting subclinical, possibly infectious, orchitis (table).

Studies of the families of patients with testis cancer show a 6–8 fold increase in risk of developing this type of cancer. Genetic constitution is clearly a fourth dimension of interacting factors. Data from a recently published genome microsatellite typing study of 35 families,[3] found at least three possible linkage clusters with LOD score greater than

Testis size	Adult mumps	Fluctuating testis pain	Raised FSH	Raised FSH and testis pain
Small (n=13) (<10 mL)	15%	54%	85%	46%
Median (n=13) (10–15 mL)	nil	31%	54%	8%
Large (n=12) (>15 mL)	nil	8%	25%	nil

FSH=follicle stimulating hormone.

Table: **Fluctuating testicular pain and testis atrophy in germ cell tumour patients**