Human leucocyte antigens and nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) affects the epithelium lining the nasopharynx. The tumour occurs most often in the Southern Chinese, at an incidence rate of 30-50 per 100 000 people/year, in contrast with <1 per 100 000 people/year in white Europeans [1-4]. It is especially common in Guangdong Province, China. The incidence rate persists in the Southern Chinese who migrated to Singapore, Malaysia, Taiwan, Hong Kong and the U.S.A. [5-7]. The disease also occurs moderately often (3-10 per 100 000/year) in several non-Chinese ethnic groups, such as Malays, Thais, Vietnamese, the Inuit (Eskimos) and Maghrebian Arabs of North Africa [8, 9].

Studies of NPC show a combination of predisposing or risk factors, including the patients' human leucocyte antigen (HLA) type, a male predominance of 2.5 to 1, infection by the Epstein-Barr virus (EBV), and a range of carcinogens [10-12]. EBV was the candidate causal factor first identified in NPC [13]. An elevated serum titre of both IgG and, in particular, IgA to viral capsid antigen and early antigen (Ea) is characteristic of NPC [14, 15]. However, as EBV infection is common and often occurs in early childhood, it is unlikely to be the main determinant of the disease. Nonetheless, an elevated anti-Ea IgA titre is a valuable diagnostic aid.

Other risk factors are environmental carcinogens, e.g. in cigarette- and wood-smoke, and in certain foods. For example, in a recent comparison of 200 Singaporean Chinese adults with NPC and 406 control subjects, an increased risk for NPC was linked to a high intake of salted soya beans, salted mustard greens and canned pickled vegetables, and a low intake of vitamin E [16]. This result confirms observations in Hong Kong and China that salted fish, duck eggs and mustard greens contain carcinogens linked to NPC [17].

Whereas all these risk factors are supported by ample data, the most compelling evidence comes from a combined HLA study of 30 sibling pairs from China, Hong Kong, Singapore and Malaysia with more than one case of NPC in each family [18]. There was a highly significant association of NPC with shared HLA haplotypes between affected sibs, which suggests the existence of an NPC disease susceptibility gene (NPC-DSG) close to the HLA region. A genetic model favouring a recessive gene predicts a relative risk of 20.9 for a homozygous subject, with 95% confidence limits of 5.1 to infinity. The identity of the NPC-DSG gene and how it interacts with the other risk factors are unknown. The present article focuses on the relation of HLA-associated genes to NPC.

HLA haplotypes

HLA-A2/B46. A possible association of HLA with NPC first arose in a study in Singapore, which showed that the disease correlated with HLA-A2 and a B-locus blank [19]. The latter was later identified as the B46 antigen. The linkage between the A2/B46 haplotype and NPC was substantiated in several studies carried out in Chinese patients in Malaysia, Hong Kong, China and California, U.S.A. (see [5] for example). How the A2/B46 haplotype confers susceptibility to NPC is unknown.

As the physiological function of HLA is antigen presentation, one hypothesis is that these particular HLA Class I antigens may not present EBV antigens sufficiently, thereby allowing the EBV to persist in nasopharyngeal epithelial cells — the hypothesis of viral persistence. Indeed, EBV persistence is linked to several other cancers such as Burkitt's lymphoma, Hodgkin's disease, and some B-cell lymphomas [12]. One candidate antigen is the EBV LMP-2 gene product, which HLA-A2.1 (A*0201) restricts in the generation of a cell-mediated response [20]. As 95% of white Europeans have the A*0201 compared with only 50% of Chinese subjects, EBV may persist more often in the Chinese, thereby supporting the model [21].

However, by applying high-resolution HLA typing of A2, we showed that A*0201 was present in 39.5% of NPC cases compared with only 15.7% of normal control subjects, the reverse of what the model predicted [22]. Therefore, A*0201 is unlikely to be a factor in the EBV clearance hypothesis, at least not in relation to LMP-2. Another observation...
was the strong negative association of the A*0207/non-B46 haplotype with NPC ($P=0.001$). The result implies a protective role for this haplotype, since it is completely absent from NPC patients [22]. Our team is working on identifying genes in this A2/non-B46 group which may explain its protective effect. There is thus no firm evidence for the idea that the antigen presenting capacity of A2 or B46 fully explains the genetic susceptibility of individuals to NPC. As only one antigen system (LMP-2) has been studied, there is scope to define other viral or tumour antigen peptides which may correlate with the disease.

**HLA-B58.** Another HLA antigen which is associated with NPC is B58, which occurs in both Chinese and Malay patients with NPC [23]. Patients bearing B58 are younger (<30 years) than those bearing A2/B46. As there is no clear bimodal pattern in NPC incidence, however, the age relation with B58 is unhelpful. More importantly, B58 appears to mark a poor prognosis, as it occurs less often in long-term survivors of the disease [24].

**HLA in non-Chinese patients with NPC.** At an incidence of 0.4—2.0 per 100 000 people/year, NPC is rare in white Europeans [25, 26]. Most studies in Europeans thus investigate the familial aggregation of disease, which crudely estimates the strength of haplotype association. In one such study, NPC was found in five members over three generations of a Scandinavian—American family [27]. Three of the five NPC cases had A1/B37/DR6 haplotype; the others were not HLA typed. Such data further support the existence of an NPC-DSG in the HLA region and show that HLA molecules per se are not crucial.

**T-cell receptor genes**

The development of the host T-cell repertoire is another mechanism by which the HLA system can affect susceptibility to NPC. The influence of HLA on both positive and negative selection of thymic T-lymphocytes bearing selected T-cell receptor (TCR) V genes is established [28–30]. Preliminary results from our laboratory showed that several TCR V gene families, Vx10, Vx11, Vx13, Vx14, Vβ14 and Vβ20, were absent from both the tumour and the peripheral blood of NPC patients [31]. This result implies that certain TCRs may be important in the generation of the immune response to EBV or tumour-associated antigens. Therefore, individuals lacking these TCRs may be susceptible to the disease. Six out of eight NPC patients with B46 had no detectable Vx17 compared with 6/6 normal B46 subjects. Also, Vx18 was undetectable in 5/8 NPC patients compared with 5/5 normal subjects [31]. Thus, certain HLA-TCR combinations may be sensitive to particular diseases, but whether the TCR families are eliminated before or after the disease has established itself is unknown.

These results may explain the reduction in cell-mediated responses [32, 33] and defective T-cell responses to specific EBV antigens in NPC patients [34]. Furthermore, CD8+ T-cells occur in large numbers in the peripheral blood in North African patients with NPC [35], but these CD8+ cells failed to kill HLA-matched EBV-transformed B-cells, showing that the immune response to NPC is not simple. Any construct of the immune response in NPC must include HLA partner molecules, such as TCRs.

**Tumour suppressor gene deletions**

A recent report suggests that deletions in the p16 tumour suppressor gene in NPC patients may also have a causative role [36]. How this suppressor gene may relate to the other predisposing factors is unknown.

**Concluding comments**

The study of NPC remains a challenging task. Although the risk factors are well identified, how they interact to precipitate the disease remains unknown. The task is hampered by a lack of valid in vitro models and difficulty in generating cell lines from the carcinoma. Taking into account the available data, the contribution of risk factors is weighted as follows: genetic makeup (disease susceptibility gene?) > exposure to carcinogens > EBV infection. Recent work suggests involvement of p16 tumour suppressor deletions in NPC, and further experiments may integrate this factor with the other predisposing mechanisms in this intriguing disease.

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**References**

Erythropoietin dysregulation in renal failure and research on IgA nephropathy

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Introduction
Recent research in nephrology in Singapore has had two major themes. The first is the role of erythropoietin in the anaemia which complicates chronic renal failure; the second concerns the dominant cause of primary glomerulonephritis in Singaporean patients, IgA nephropathy.

Erythropoietin dysregulation in renal failure
Erythropoietin (EPO), a glycoprotein hormone produced principally in the kidneys, is the major humoral regulator of the production of erythrocytes. In patients with chronic renal failure, anaemia is common and mainly due to a relative deficiency of EPO. Some observations suggest that the deficiency is due not to decreased production of EPO in the diseased kidneys but to abnormal regulation of EPO. Firstly, whereas baseline serum EPO concentrations are consistently and inappropriately low for the degree of anaemia in patients with chronic renal failure, serum EPO increases substantially in dialysis patients in hypoxaemia [1]. Secondly, in those patients with end-stage renal failure who develop erythrocytosis after successful renal transplantation, EPO concentration is significantly higher in the native renal vein [2].

The development, by Ratcliffe et al. [3], of a quantitative RNase protection assay to measure organ concentrations of EPO mRNA allows closer study of this important issue. Rats rendered chronically uraemic by five-sixth nephrectomy have ana-