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Does genotype mask the relationship between psychological factors and immune function?

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Abstract

This paper examined the interaction between genetic influences of the polymorphic human leukocyte antigens (DRB1 and DQB1) and psychological distress on the development of cellular immunity to the novel antigen, keyhole limpet hemocyanin (KLH). Participants ($n = 227$) were immunized with KLH and the development of cutaneous delayed-type hypersensitivity (DTH) against KLH was examined 3 weeks later. Distress was assessed using the Profile of Mood States. DNA was typed for the serologically defined DRB1 and DQB1 antigens. There was a significant correlation between distress at immunization and the development of DTH skin test responses to KLH ($n = 214$, $r = .24$, $p = .003$). HLA DQ2 was weakly associated with a decreased likelihood of developing a cutaneous delayed-type hypersensitivity response against KLH (odds ratio [OR] = 1.6; confidence interval [CI] 0.9–2.7). HLA DQ5 was weakly associated with an increased likelihood of responding to the antigen (OR = 0.6; CI = 0.3–1.0). The correlation between distress and immune function in *HLA DQ2* negative individuals was .34 ($n = 136$, $p = .00$) and in *HLA DQ2* positive individuals it was .06 ($n = 74$, $p = .64$). For *HLA DQ5* negative individuals the correlation was .26 ($n = 140$, $p = .00$) and for *HLA DQ5* positive individuals it was .22 ($n = 70$, $p = .07$). These results suggest that the distress/immune relationship in genetically susceptible or protected individuals may be underestimated in psychoneuroimmunology research.

Keywords: Keyhole limpet hemocyanin (KLH); Psychoneuroimmunology; Distress; HLA

1. Introduction

Previously we have reported considerable individual differences in the development of delayed-type hypersensitivity (DTH) skin test responses to the antigen keyhole limpet hemocyanin (KLH) (Smith et al., 2004a). Individual differences that may account for this variability in cellular immunity include the level of psychological distress experienced by participants (Smith et al., 2004a,b) and health behaviours (specifically, alcohol consumption) (Smith et al., 2004b). The research reported here examines the potential relationship between genotype, another individual difference variable, and the development of DTH responses against KLH. In particular, we were interested in the possibility that some individuals are genetically programmed to develop such robust or such weak immune responses against an antigen, that the modulatory influences of psychological factors would be difficult to detect. Indeed, it may be that genetic factors mask the role of psychological factors in psychoneuroimmunology (PNI) research. It may also be possible to identify individuals at "high risk" of failing

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to develop immunity to an antigen because of their psychological and genetic profile.

We investigated the role of the genes encoding the major histocompatibility complex (MHC), referred to in humans as the human leucocyte antigens (HLAs). MHC molecules are involved in presentation of intracellular (class I) and extracellular (class II) antigens to $CD4^+$ T cells. The focus of this research was on the MHC class II molecules that process extracellular antigens, such as KLH. When antigen-derived peptides bind with the MHC molecule they are held within a "groove" that has characteristic shape, charge, and hydrophobicity determined by hypervariable regions of the MHC encoding genes (Barber and Parham, 1993). A given peptide may bind readily with one particular antigen-binding site, but less readily with another. Antigen presenting cells with the "appropriate" binding site will be more effective in displaying a peptide to $CD4^+$ T cells and hence will mount more vigorous immune responses. Cells with a less "good fit" between antigenic fragment and antigen binding site will mount less effective responses. This "determinant selection model" (Lechler, 1994) is one explanation for the association of HLA genes with susceptibility to a number of infectious diseases (notably, leprosy, tuberculosis, persistent hepatitis, HIV, HTLV, and malaria) (Hill, 2001).

Three class II HLA proteins (HLA-DP, HLA-DQ, and HLA-DR) are encoded on the short arm of chromosome 6 which is the most polymorphic region in the human genome (So, 1994). Interest in this research focussed on the better characterized DR and DQ antigens. There is only one *HLA-DRA* gene and it displays very limited polymorphism (Das et al., 1983). The *HLA-DRB* genes are extremely polymorphic, and determine the functional characteristics of the DR molecules (She, 1996). There are nine *HLA-DRB* genes (*HLA-DRB1-9*), but only *HLA-DRB1* is invariably expressed (She, 1996; So, 1994). *HLA-DQA1* and *HLA-DQB1* are also expressed and encode polymorphic HLA-DQ chains (So, 1994). *HLA-DQA1* and *HLA-DQB1* are very tightly linked (approximately 99%) so typing for both provides very little extra information.

In this study, the role of the *HLA-DRB* and *HLA-DQB* genes in the control of responsiveness to KLH was examined. The emphasis, however, was on the implications of genotype for the detection of relationships between psychological factors and immune function.

2. Material and methods

2.1. Protocol development

2.1.1. Subjects

Two hundred and twenty-seven healthy students (124 males and 103 females) participated. All but five (three from the engineering and two from the arts faculties) were medical students. Sixteen were involved in the protocol development stage of this research, 45 were recruited for an investigation of distress/immune relationships (Smith et al., 2004a) and a further 166 recruited for an extension of that study (Smith et al., 2004b). In the latter two studies, distress was related to a reduced capacity to develop DTH skin test responses against KLH. Ages ranged from 17 to 31 years ($M = 20.4$, $SD = 2.1$).

This research was approved by the Committee on Experimental Procedures Involving Human Subjects of the University of New South Wales. Informed, written consent was obtained from all subjects before the commencement of procedures. Subjects were excluded from the study on the basis of: (i) seafood allergy; (ii) pregnancy; (iii) significant illness or operation in the past month; (iv) a history of a major immune-related illness (for example, diabetes or cancer); (v) immuno-suppressive medication or (vi) intercurrent infection at the commencement of the study.

2.2. Immunization protocol

The development of this protocol is described elsewhere (Smith et al., 2004a). Subjects were immunized with 0.1 mg KLH (1.25 mg/ml) adsorbed to 0.9 mg alum (45 mg/ml) administered into the deltoid muscle.

2.3. Assessment of KLH-induced DTH skin responses

To assess DTH responses 0.001 mg KLH in 0.01 ml saline solution was administered as an intradermal injection into the volar aspect of the arm. At 48 h after administration, the response was determined by finding the mean of any resulting induration in millimetres for two diameters at right angles. A positive response was defined as a mean diameter of greater than 2 mm (Hortobagyi et al., 1981). Participants were trained to read their own DTH responses, although all but five of the participants were medical students who had prior experience with the procedure. The subjects were instructed to ignore any erythema and to identify the area of induration by carefully approaching the immunization site with a ballpoint pen. Typically, the pen stops at the edge of the induration (McLean, 1988).

2.4. DNA extraction

DNA was extracted from whole blood ($n = 166$) or from PBMCs cryopreserved on liquid nitrogen ($n = 61$). DNA extractions were carried out by a standard salting out method (Miller et al., 1988) using ammonium acetate after proteinase K treatment. DNA was then precipitated out with ethanol and resuspended in TE (10 mM Tris, 1 mM EDTA, pH8.0) buffer.