Genetics of the immune cell receptors TCRB and CCR5 in human disease

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DECLARATION

Declaration

I hereby declare that the research data presented in this thesis is the result of original research conducted by myself, except where otherwise acknowledged. This thesis has not previously been submitted for a degree at this or any other university.

Marc McWilliams Buhler

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Abstract

Early in the evolution of the vertebrates it is thought that two genomic duplications occurred, providing a basis for the evolution in body plan and neural crest of very early vertebrates and substantive material for further evolution of various gene families such as those making up a number of components of the adaptive vertebrate immune system. While the bony fish possibly had another, genome duplications are not generally a feature of vertebrate evolution and indeed the appearance of an antigen-adaptive immune recognition system may have served to limit the size that various vertebrate genomes, including that of the human, can in fact achieve. This initial step in vertebrate immune evolution, the establishment of recognition of non-self against the unique set of "self" epitopes for an individual, provided an immensely powerful weapon in immune function with the ability to tailor a defense against as-yet-unseen dangers at any time albeit with the pitfall of autoimmune disease. As the recognition sites of the antigen receptor molecules such as TcR are produced by clonal modification of the segments provided in the germline and are thus not in the genome itself, pathogens have not been able to hijack this one component of the immune system in the way so many other components have been put to use throughout evolution, nor do these components necessarily reveal themselves as associated with disease through genome screens. Importantly, overall immune function is determined not just by the potential repertoire of recognition receptors but also by the ability of immunocompetent cells to migrate in a tissue specific fashion through the use of various chemokines and their receptors. Typical of the hijacking of an immune system component by a pathogen is the use of a chemokine ligand gene in the viral ancestor to SIV and HIV, allowing for virus binding to immunocompetent cells as is seen in the use of the CCR5 chemokine receptor by macrophage-tropic HIV strains.

This thesis describes the allele and genotype frequencies for several TcR beta-chain variable segment polymorphisms in a population of MS patients compared with controls

before and after stratification for HLA-DR15, polymorphism in the Apo-1 / Fas promoter, the DRB1 Val^{86}/Val^{86} genotype, *CCR5-Δ32* and the HLA-DRA promoter. The thesis continues with *CCR5-Δ32* genotyping in IDDM, MS and SLE cohorts and then examines the question of the population of origin of the delta-32 allele of the CCR5 receptor for chemokine. Here, a case / control comparison of 122 RR-MS patients with 96 normal individuals was made for allele and genotype frequencies and for haplotypes formed by pairs of TCRB markers. Further analysis was made after HLA-DR15 stratification. Linkage disequilibrium was found between pairs of alleles of bv8s1, bv10s1, bv15s1 and bv3s1 loci in both patients and controls. In the RR-MS cohort, an increase in the allele frequency of bv8s1*2 was seen ($p = 0.03$) and the haplotype bv8s1*2 / bv3s1*1 was increased ($p = 0.006$), and both were found to be statistically significant. In the DR15-positive group, association between MS and TCRB was seen with the bv8s1*2 allele ($p = 0.05$) and the bv8s1*2 / bv10s1 haplotypes ($p = 0.048$), while the haplotype associations seen among the DR15-negative patients included the bv3s1*1 allele (bv10s1*1 / bv3s1*1, $p = 0.022$; bv8s1*2 / bv3s1*1, $p = 0.048$). While no associations were found after stratification for SDF1-3'A, Apo-1 / Fas or DRB1 there were modest interactions between bv3s1, bv10s1 and bv15s1 and the HLA-DRA promoter. These results support the involvement of the TCRB region in MS susceptibility.

The further study of autoimmune disease here includes genotype analysis of *CCR5-Δ32* in type 1 diabetes (IDDM) and SLE. CCR5 is the major co-receptor for viral entry used by macrophage-tropic HIV strains and protection from infection is seen in homozygotes for *CCR5-Δ32.* In diabetes, infiltration of pancreatic tissue by autoreactive T-cells involves secretion of multiple cytokines and chemokine receptor expression. Variation in the chemokine receptor CCR5 may result in differences in inflammatory cell migration in response to relevant chemokines. Adolescents with type 1 diabetes were genotyped for *CCR5-Δ32* ($n = 626$). The allele frequency was compared with that of

253 non-diabetic adolescents and with that of 92 adults with SLE. A reduced allele frequency was seen in type 1 diabetes compared with controls (0.092 vs 0.123, p = 0.05). This difference was not seen for the cohort of patients with SLE (freq = 0.114). A reduction in the number of *CCR5-Δ32/Δ32* homozygotes, who lack CCR5, in the type 1 diabetes cohort was also seen and while not statistically significant (2 observed compared to 5.25 expected; p = 0.12) is interesting. These results suggest a partial protection from type 1 diabetes for *CCR5-Δ32* homozygous individuals is possible and that CCR5 has a potential role in the pathogenesis of type 1 diabetes.

Global surveys of the *CCR5-Δ32* allele have confirmed a single mutation event in a Northeastern European population as the source of this allele. Here, Australian Ashkenazi Jews (n = 807) were found to have a *CCR5-Δ32* allele frequency of 14.6% while Australian Sephardic Jews (n = 35) had a frequency of 5.7% and non-Jewish Australian controls (n = 311) had an allele frequency of 11.25%. Data on birthplace of grandparents showed a gradient with highest *CCR5-Δ32* frequencies from Eastern European Ashkenazim (~19.5% for those whose four grandparents come only from Russia, Poland, Hungary, Austria and Czechoslovakia; n = 197) which differs significantly from the frequency seen in Ashkenazi Jews from Western Europe (n = 101, p = 0.001). Homozygotes for *CCR5-Δ32* were genotyped with 3p21 region microsatellites. This has defined an ancestral haplotype on which the mutation first occurred and helped to date this event to between 40 and 50 generations ago or just over a thousand years ago. The population gradient, combined with the dating of the mutation by microsatellite allele frequencies, suggests an origin for the *CCR5-Δ32* allele in a population ancestral to the Ashkenazim. The distribution in non-Jewish populations in northern Europe has led others to postulate spread of the mutation by Vikings. It is hypothesised here that the link between the two populations could be the kingdom of Khazaria with subsequent admixture into both Swedish Vikings and Ashkenazi Jews.

The basic driving force of evolution is through selection and the immune system has a role which, through the survival pressure exerted by viruses and other pathogens, has the potential to exert a great deal of selective force on the various components of this system. The effects of this pronounced selection on an immune system component can be seen for example in the increase of the *CCR5-Δ32* allele over the last thousand years to the current frequency. As mentioned, some immune system components are not affected by such straightforward selection. In the case of the TCRBV segments, effects on the immune repertoire can occur through MHC interaction at the point of thymic entry and in the effects of various superantigens, but the actual binding pockets that recognise antigen are themselves unable to be selected for (or against). The findings presented in this thesis provide support for the association of TCRBV gene segments with multiple sclerosis and also provide support for the further study of the role of the *CCR5-Δ32* allele in type 1 diabetes. Furthermore, data presented here suggests that the *CCR5-Δ32* allele had an origin in the Khazar Kingdom just over a thousand years ago, accounting for the allele frequencies in both the Ashkenazi Jews and in lands frequented by the Vikings. The definition of an extended ancestral haplotype for the *CCR5-Δ32* allele shows how the effect of selection of an allele of one gene can carry with it specific alleles of a large number of other genes as well.

Publications arising from work contained in this thesis

Chapter 3

Buhler, M. McW., BH Bennetts, RNS Heard, GJ Stewart (2000). T cell receptor β chain genotyping in Australian relapsing-remitting multiple sclerosis patients. Multiple Sclerosis 6:140-147.

This paper contains the data presented and discussed in chapter 3.

Teutsch, SM, QR Huang, M. Buhler, BH Bennetts, RNS Heard, N Manolios, GJ Stewart (2000). Evaluation of the Apo-1/Fas promoter Mva 1 polymorphism in multiple sclerosis. Multiple Sclerosis 6:14-18.

In this paper, the TCRBV genotyping was used for stratification and assessment of potential genetic interactions between the T-cell receptor and the apo-1/Fas gene.

Teutsch, SM, BH Bennetts, MM Buhler, RNS Heard, GJ Stewart (1999). The DRB1 Val86/Val86 genotype associates with multiple sclerosis in Australian patients. Human Immunology 60:715-722.

In this paper, the TCRBV genotyping was used for stratification and assessment of potential genetic interactions between the T-cell receptor and DRB1 Val/Gly aa 86 polymorphism.

Bennetts, BH, SM Teutsch, MM Buhler, RNS Heard, GJ Stewart (1999). HLA-DMB gene and HLA-DRA promoter region plymorphisms in Australian multiple sclerosis patients. Human Immunology 60:886-893.

In this paper, the TCRBV genotyping was used for stratification and assessment of potential genetic interactions between the T-cell receptor, HLA DMB and HLA-DRA promoter genes; modest interactions were noted between the TCRBV3S1, TCRBV10S1 and TCRBV15S1 loci and HLA-DRA promoter genotypes.

Bennetts, BH, SM Teutsch, MM Buhler, RNS Heard, GJ Stewart (1997). The CCR5 deletion mutation fails to protect against multiple sclerosis. Human Imm. 58:52-59.

In this paper, the TCRBV genotyping was used for stratification and assessment of

potential genetic interactions between the T-cell receptor and CCR5-Δ32 mutation.

Chapter 4

Buhler, MM, M. Craig, KC Donaghue, P Badhwar, J Willis, N Manolios, BD Tait, M Silink, BH Bennetts, GJ Stewart (2002). CCR5 genotyping in an Australian and New Zealand Type 1 diabetes cohort. Autoimmunity 35:457-461.
This paper contains the data presented in Chapter 4.

Chapter 5

Buhler, M McW, A Proos, V Howell, BH Bennetts, L Burnett, GJ Stewart (submitted). Evidence of an Eastern European Ashkenazi Jewish Enrichment of the CCR5-delta32 allele: a clue to the origin? *This paper contains the data presented in Chapter 5*.

Naif, HM, AL Cunningham, M Alali, S Li, J Nasr, MM Buhler, D Schols, E de Clercq, GJ Stewart (2002). A human immunodeficiency virus type 1 isolate from an infected person homozygous for CCR5-Δ32 exhibits dual-tropism by infecting macrophages and MT2 cells via CXCR4. Journal of Virology 76:3114-3124.
This paper, aimed at identifying the co-receptor used in an HIV infected CCR5-Δ32 homozygote, involved the use of the microsatellite assays in the CCR5 region developed and described in Chapter 5 as well as microsatellite markers for CXCR4. The microsatellite data carried out by were critical for the identification of genetic identity between this individual and other family members studied.

Abstract accepted for oral presentation:

Marc M. Buhler, Anné Proos, Viive Howell, Bruce H. Bennetts, Leslie Burnett and Graeme J. Stewart. Could admixture of the *CCR5-Δ32* allele into Ashkenazi Jews and Vikings be explained by an origin in the Kingdom of the Khazars?
This abstract was presented as a talk in the symposium on Empirical Population Genetics at the XIX International Congress of Genetics, Melbourne, 2003.

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Abbreviations

Δ32	32 basepair deletion (delta 32) mutation of CCR5
ag	antigen
AIDS	acquired immune deficiency syndrome
APC	antigen presenting cell
blast	basic local alignment search tool
blat	basic local alignment tool
bp	basepair
contig	“contiguous” joining of adjacent sequences of DNA
cM	centimorgan
CP-MS	chronic-progressive MS
CNS	central nervous system
DC	dendritic cell
IDDM	type 1 diabetes (prev. insulin dependent diabetes mellitus)
EBV	Epstein-Barr virus
FCS	fetal calf serum
HCMV	human cytomegalovirus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
Ig	immunoglobulin
Kbp	kilobasepair (1,000 bp)
LCL	lymphoblastoid cell line
LD	linkage disequilibrium
Mbp	mega-basepair (one million bp)
MCMV	murine cytomegalovirus
MHC	major histocompatibility region
MIP-1α	macrophage inflammatory protein-1α
MS	multiple sclerosis
OND	other neurological disease
PAGE	polyacrylamide gel electrophoresis
PBMC	peripheral blood mononuclear cell

PCR	polymerase chain reaction
RA	rheumatoid arthritis
RR-MS	relapsing-remitting multiple sclerosis
RP-MS	relapsing / progressive MS (secondary-progressive MS)
T1D	type 1 diabetes
TcR	T-cell antigen receptor
TCRB	T-cell receptor (beta) variable segment
TCRBV	T-cell receptor (beta) variable segment
Th1	helper T-cell type 1
Th2	helper T-cell type 2
SLE	systemic lupus erythematosus