Workshop

The Role of HLA-B27 in Spondyloarthritis

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ABSTRACT. This article summarizes the proceedings of a one-day international workshop held in July 2009 on the role of HLA-B27 in the pathogenesis of ankylosing spondylitis (AS) and related disorders. HLA-B27 is found in about 90% of patients with AS, with an odds ratio of about 100, but the mechanism underlying this association is not known. There are currently 3 major mechanistic hypotheses for this association: (1) T cell recognition of one or more B27 presented peptides; (2) B27 heavy-chain misfolding that induces an unfolded protein response; and (3) innate immune recognition of cell-surface expressed B27 heavy-chain dimers. None of these hypotheses accounts for the tissue specificity of the inflammation characteristic of AS. These hypotheses were discussed in the context of known epidemiologic, biochemical, structural, and immunologic differences among HLA-B27 subtypes; data from the HLA-B27 transgenic rat model of spondyloarthritis; the growing list of other genes that have been found to be associated with AS; and other data on the pathogenesis of spondyloarthritis. Proposed directions for future research include expanded efforts to define similarities and differences among the B27 subtypes; further development of animal models; identifying the interactions of B27 with the products of other genes associated with AS; and continued investigation into the pathogenesis of spondyloarthritis. (J Rheumatol 2010;37:2606–16; doi:3899/jrheum.100889)

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On July 23, 2009, several dozen investigators gathered in Houston, Texas, for a one-day discussion workshop on human leukocyte antigen-B27 (HLA-B27). The event, funded by the US National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), was held in conjunction with a 3-day joint conference, organized by John Reveille (University of Texas, Houston), of several groups: the annual meeting of Spondylitis Research and Therapy Network (SPARTAN), a working meeting of International Genetics of Ankylosing Spondylitis (IGAS), and interested members of the Pan American League of Associations of Rheumatology (PANLAR). The dramatic association between HLA-B27 and ankylosing spondylitis (AS) was first reported in 1973, but the molecular basis for this association remains unknown. For several decades, research on HLA-B27 featured prominently at meeting sessions and in the literature on spondyloarthritis (SpA). In recent years, however, the focus has shifted to anti-tumor necrosis factor (TNF) therapy, magnetic resonance imaging, and genome-wide genetic studies, among other areas. As a result, the need arose for a separate, extended meeting session dedicated to the still fascinating and highly relevant question of how HLA-B27 confers susceptibility to SpA. The workshop brought together most of the investigators who work on HLA-B27, along with others with expertise in major histocompatibility complex (MHC) biology or other relevant areas. The day was divided into 4 sessions, each introduced by one or 2 brief overview presentations, followed by a lengthy general discussion. A number of themes recurred throughout the sessions, and to avoid unnecessary repetition, this summary is organized around these themes, rather than strictly by session topic.

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Epidemiology of the HLA-B27 Subtypes

This theme was introduced by Carlos López-Larrea (Central Hospital of Asturias, Oviedo, Spain). HLA-B27 was first defined by alloantisera, like all of the HLA class I alleles described in the 1960s and 1970s[1]. Since then, 72 HLA-B27 subtypes (alleles) have been described at the protein sequence level[2], 27 of these since January 2009. The major subtypes that have been shown in large epidemiologic studies to be associated with AS are HLA-B*2705 (Caucasians and worldwide), HLA-B*2704 (Far East), HLA-B*2702 (Mediterranean Caucasians), and to a lesser extent HLA-B*2707 (South Asians, Middle East)[3]. HLA-B*2703 was originally described in sub-Saharan Africa, but has recently been reported in both AS patients and healthy B27+ controls in central China[4]. More anecdotal associations with AS have been reported for HLA-B*2701, -08, -10, -13, -14, -15, -19, -23, -24, and -25[5]. Most of the subtypes are too rare

and too recently described to have been evaluated for disease association.

Of particular interest are 2 subtypes, HLA-B*2706 and HLA-B*2709, that have been reported to lack association with AS. B*2706 is a common B27 subtype in Southeast Asia. HLA-B*2709 is a much rarer subtype, found primarily on the island of Sardinia. In López-Larrea's summary of the literature, B*2706 has been reported in 100 of 1313 healthy B27+ controls, compared with 4 out of 676 AS patients ($p = 2.6 \times 10^{-11}$), with no clinical information reported for these 4 AS patients. B*2709 in Sardinia, southern Italy, and Tunisia has been reported in 33 of 312 healthy B27+ controls, and in only 1 of 493 AS patients ($p = 8 \times 10^{-9}$). In addition, B*2709 has been reported anecdotally in 2 patients with AS, but each had another predisposing factor[5]. The general consensus is that these 2 subtypes show a much lower predisposition to AS, compared with the other subtypes found in these populations. Whether this can be attributed to biochemical and/or immunological properties of the molecules themselves was the topic of considerable discussion. One confounding factor is the finding that these subtypes tend to reside on HLA haplotypes that differ from those of known disease-prone B27 subtypes[6]. Other genetic or environmental factors in certain populations may also play a role such that AS is not found even among individuals with susceptible B27 subtypes. Examples include lack of AS in individuals in The Gambia with B*2705 or B*2703[7] or Greek Cypriots with B*2707[8]. Even among established disease-associated subtypes, a hierarchy of susceptibility has been observed. Specifically, in Asian populations, B*2704 seems to confer greater susceptibility than B*2705[9]. In Caucasian populations, B*2705 and B*2702 appear to confer equal susceptibility in numerous studies.

There was some discussion of HLA-B*1402 and HLA-B*1403. These 2 alleles differ from one another only at position 156. B*1403, which is very rare, has been associated with AS in 2 small series from Africa[10,11], with a relative risk for AS comparable to that of B27. Dr. Reveille reported that in his group's study of African Americans, B*1403 was found in only 1 of 350 controls, and in none of 55 patients with AS. Although B*1403 differs from B*2705 at 18 positions, and B*1402 at 17, both share the unpaired Cys67 residue with the consensus B27 sequence. B*1402 is found in African and Caucasian populations, and shows no association with AS.

Biochemistry and Peptide Repertoires of the B27 Subtypes

This theme was introduced by José López de Castro (Severo Ochoa Center for Molecular Biology, Madrid, Spain). He and his colleagues have isolated and sequenced peptides bound to several different subtypes, and have also examined the cross-reactivity of alloreactive T cell clones raised against particular subtypes. They have found a general correlation between peptide sharing and the degree of shared T cell reactivity. For example, B*2705 and B*2709, which differ by only one amino acid, share 79% and 88%, respectively, of the peptide repertoires isolated from the 2 molecules, and are recognized by 80% and 90%, respectively, of the alloreactive T cell clones generated against the 2 molecules[12,13]. In contrast, B*2706 and B*2707, which differ by 5 amino acids, share 39% of their peptides and 21% T cell cross-reactivity. Peptide differences between subtypes reside mainly in C-terminal residues and/or secondary anchor positions.

B*1402 and B*1403 share only about 30% of peptides and T cell cross-reactivity, despite the difference of only 1 amino acid. B*2705 and B*1403 show only 3%–5% peptide sharing[14]. Interestingly, a single peptide, IRAAPPPLF, derived from cathepsin A signal sequence residues 2–10, binds B*2705, B*2709, B*1402, and B*1403, and a crystallographic study of the first 3 alleles (B*1403 crystals could not be obtained) showed that all bind this peptide with the same conformation[15]. This peptide binding evidently might account for the very limited alloreactive cytotoxic T lymphocytes (CTL) cross-reactivity observed among B*2705, B*1402, and B*1403[14].

B27 subtypes differ substantially in their rate of assembly and folding in the endoplasmic reticulum (ER) and in the stability of the heavy-chain (HC)-ß$_2$-microglobulin peptide complexes. The disease-prone subtypes B*2705, -04, and -02 show very prolonged assembly kinetics, with folding times up to 30-fold longer than for the control allele HLA-B7, and 5- to 10-fold longer than for B*2706 and -09. Once assembled, these complexes show more stability, as measured by ~2-fold longer survival at 50°C than -06 or -09. Peter Cresswell (Yale University, New Haven, CT, USA) pointed out that this suggests more stringent quality control for acquisition of the peptide repertoire in the ER. This is also consistent with the strong association of ß$_2$-microglobulin (ß$_2$m) with B*2705 and -02[16]. However, slow assembly and high thermostability do not correlate with disease predisposition, since B*2707 shows more rapid assembly and lower thermostability, similar to -06 and -09. In these 6 most heavily studied naturally occurring B27 subtypes, the correlation of rate and assembly and thermostability is with position 116 in the F pocket that interacts with the peptide C-terminus (Asp in B*2702, -04, and -05; Tyr in -06 and -07; His in -09). This correlation is not maintained in site-directed mutants.

A constant feature of all the B27 subtypes studied to date is an almost absolute requirement for Arg at position 2 of the bound peptide[17]. B*1402 and B*1403 both show a strong, but not absolute, preference for Arg at P2[14]. HLA-B*3901, which has been reported in B27-negative Japanese AS patients and which shares the B pocket residues Cys67 and Glu45 with the consensus B27 sequence, also accommodates Arg at P2[18]. Gorillas with SpA have been shown to

share an allele, GogoB*0101, at the MHC locus homologous to HLA-B[19]. This allele shares many polymorphic residues with HLA-B*2702, but most of its B pocket is dissimilar to B27. Nonetheless, it also shows a predominance of Arg at P2 of eluted peptides, and *in vitro* capacity to bind known B27 peptides. Dr. Reveille pointed out that another HLA-B allele, B*4001 (a subtype of HLA-B60), has shown association with AS in several different populations with an odds ratio (OR) of 1.3 irrespective of the presence of B27. This allele has Ser67 but lacks several other features of B*2705, -04, and -02, and its peptide repertoire has not been well studied. Other HLA-B alleles that showed association with AS in the population studies described below by Matthew Brown (University of Queensland, Brisbane, Australia) include B*38 and B*52. Which subtypes of these alleles are associated is not yet known.

Recent work by Arie Admon (Technion, Haifa, Israel), using more powerful mass spectrometry and software, has significantly expanded the database of B*2705-bound peptides[20,21]. Application of this methodology to comparisons among subtypes, between patients and controls, and among different cell sources, should provide new insight into the role of B27 in disease. Dr. Admon was unable to attend the workshop.

T Cell Recognition of HLA-B27

This theme was introduced by Rosa Sorrentino (University La Sapienza, Rome, Italy). Once it became clear from the 1987 description of the HLA-A2 crystal structure that MHC molecules present peptides, the concept that B27 causes SpA by presenting an "arthritogenic peptide" became a dominant hypothesis for the pathogenic role of B27[22]. This hypothesis has been reinforced by the data cited above, showing a correlation between the P2 Arg peptide motif and disease predisposition. Moreover, B27 is known to predominate in the CTL response to a number of viruses, and to be associated with a protective response to human immunodeficiency virus and hepatitis C virus[23,24,25,26,27,28,29,30].

Dr. Sorrentino and her colleagues have been the primary group to describe the epidemiology of B*2709 in Sardinia, and they have focused their studies of T cell recognition on peptides bound to HLA-B*2705 and B*2709. Initially, they observed that the CTL response to the Epstein-Barr virus (EBV) peptide LMP2p236-244 (RRRWRRLTV) showed different fine-specificity in B*2705 versus B*2709 donors[31]. They then showed that closely related peptide derived from the vasoactive intestinal peptide receptor 1 (pVIPR400-408; i.e., RRKWRRWHL) evoked CTL responses in B*2705+ but not B*2709+ individuals, despite binding to both alleles[32]. B*2705+ and B*2702+ patients with AS showed evidence of persistent endogenous activation of this response[32,33,34].

In collaboration with Andreas Ziegler and Barbara Uchanska-Ziegler (Charité University Hospital, Berlin, Germany), who have contributed extensively to the B27 field but were unable to attend this workshop, Dr. Sorrentino's group showed by crystallography that pVIPR is bound in 2 different conformations by B*2705 (Arg at P5 pointing down to form salt bridge with Asp116, or pointing up to solvent) but only in the latter conformation by B*2709[33]. Another peptide pGR (412-420; RRRWHRWRL) from the glucagon receptor shows a dual conformation in both B*2705 and B*2709 and evokes a CTL response in both[35,36]. This correlation between dual conformation and T cell self-reactivity is a novel finding that opens the possibility of a connection to AS pathogenesis. In response to a question, Dr. Sorrentino said they had not made any B27-peptide tetramers to look for the prevalence of specific CTL in patients or controls.

Dr. Cresswell said that a highly disproportionate amount of investigative attention has been paid to B27, compared with other HLA class I alleles, and he thought it likely that there are other alleles that also exhibit dual conformation. This theme of caution was repeated by others in regard to other unusual features of B27 that were discussed.

Dr. López de Castro and colleagues have recently demonstrated the production and presentation by B*2705 in transfected cell lines of 2 peptides from *Chlamydia trachomatis* (a trigger of reactive arthritis) that are known T cell epitopes, based on earlier work by Joachim Sieper and colleagues (Charité University Hospital, Berlin, Germany)[37,38]. In response to Dr. Cresswell's note of caution, Dr. López de Castro replied that dual conformation for an MHC I-peptide structure is indeed very rare.

The search for B27-restricted autoreactive CD8+ T cells in AS has been a long and difficult one. Robert Colbert [National Institutes of Health (NIH), Bethesda, MD, USA] pointed out that interpretation of past studies of B27-restricted CD8+ T cells, which usually employed EBV-transformed cell lines, is confounded by the strong B27-restricted T cell response to EBV antigens. It is clear from Dr. Sorrentino's work that peptide-specific B27-restricted autoreactive CD8+ T cells are not uncommon in AS patients, and continued work should help determine their significance in disease pathogenesis.

HLA-B27 Misfolding and the Unfolded Protein Response

Dr. Colbert summarized his work in rat and human systems regarding the phenomenon of HLA-B27 misfolding. His group originally observed that HLA-B*2705 in cell lines showed a fraction of heavy chains (HC) undergoing ER-associated degradation[39]. At the same time, Bowness, *et al* showed that B*2705 HC *in vitro* spontaneously formed disulfide-linked homodimers[40]. In leukocytes from B*2705 transgenic rats, particularly ones with very high gene copy number[41,42], B27 HC form easily detectable heterodimers and higher oligomers in the ER under conditions in which

B27 is upregulated[43,44]. These and possibly other misfolded forms in turn render cells susceptible to the unfolded protein response (UPR), the program of signal transduction generated by ER stress and the accumulation of unfolded or misfolded proteins[45]. The B27 HC misfolding is thought to depend on disulfide bond formation, including, but not necessarily exclusively dependent on, the unpaired Cys67[44,46].

Working predominately with B27/hß$_2$m transgenic rat bone marrow-derived macrophages, Colbert's group has shown that stimulation with TNF-α, lipopolysaccharide, and/or interferon-γ (IFN-γ) causes a marked upregulation of B27 HC expression and HC misfolding and a parallel upregulation of UPR markers[43,47]. This is associated with a marked upregulation of interleukin 23 (IL-23)[48]. The particular B27 transgenic rat line with which they were working develops severe colitis, and the IL-23 upregulation was associated with a marked increase in IL-17-producing T cells in the colon, along with modest IL-12p35 upregulation, compared with wild-type. They have also shown that with drug-induced UPR in cell lines and also in Toll-like receptor 3- (TLR3) or TLR4-stimulated B27 transgenic rat macrophages, there is a marked upregulation of IFN-ß that is dependent upon XBP-1 splicing, a major UPR signaling pathway[49]. The IL-23/IL-17 upregulation is quite intriguing, since IL-23 receptor polymorphisms have been shown to be associated with AS, psoriasis, and Crohn's disease[50,51,52]. IL-17 has been implicated in the pathogenesis of Crohn's disease and psoriasis[53,54], and evidence is growing for its involvement in AS[55,56] (and data from several groups presented at this workshop).

The misfolded B27 is recognized experimentally with the monoclonal antibody HC10[57]. Simon Powis (University of St Andrews, Fife, Scotland) asked whether HC10 recognizes any form of B27 associated with ß$_2$m and/or peptide, and also whether there are intermediately folded forms that are not seen by this antibody or by antibodies to folded B27. The consensus was that additional reagents are needed to improve understanding of the various forms of B27 in the ER.

There was considerable discussion about misfolding and the B27 subtypes. Misfolding leading to UPR activation has not been studied rigorously with subtypes other than B*2705. It may be related to slow processing in the ER described above, which is seen with B*2705, -04, and -02 but not with -06, -07, or -09, but this has not been tested experimentally. There was also discussion of the observation that B27 transgenic rats carrying additional hß$_2$m show less B27 HC misfolding but increased arthritis and spondylitis[58], apparently confounding any simple correlation between B27 misfolding and disease susceptibility. However, Dr. Colbert emphasized that the extra hß$_2$m does not completely abolish the UPR in cytokine-stimulated macrophages from these rats, leaving open the possibility that a smaller UPR might be more pathogenic than a large one.

Paul Bowness (Oxford University, Oxford, UK) asked when and where B27 triggering of the UPR might play a role in human AS. He noted that his group had not found evidence for this response in blood monocytes from the small number of AS patients they had examined. Dr. Colbert suggested that tissues involved in disease would be the best place to start. Jane Goodall (Cambridge University, Cambridge, UK) noted that in human monocytic cells and monocyte-derived dendritic cells, she and her colleagues see UPR-related upregulation of IL-23 and osteoprotegerin. TLR agonists synergize with pharmacologic stimulators of the UPR in this regard, and intracellular infection with *Chlamydia trachomatis* also activates the UPR and induces IL-23 production. The UPR-related transcription factor CHOP appears to be central to the IL-23 upregulation. They have not yet examined whether this process is enhanced by B27.

HLA-B27 Surface Homodimers and Recognition by Natural Killer (NK) and Other Receptors

Dr. Bowness presented his group's work, focusing on cellular interactions presumed to be based on cell-surface expression of B27 HC homodimers. As noted above, he and his colleagues observed that B27 HC readily form homodimers *in vitro*. This occurs in the absence of peptide but is enhanced in the presence of peptide. They also observed cell-surface expression of B27 HC homodimers in cell lines and AS patients' peripheral blood mononuclear cells (PBMC)[59]. Using a fluorescence-tagged tetramer of B*2705 HC dimers, they showed its binding to the cell surface NK inhibitory receptors KIR3DL1 and KIR3DL2, whereas only KIR3DL1 bound tetramers of conventional B27 HC with ß$_2$m and peptide. The HC-dimer tetramer also bound the leukocyte immunoglobulin-like receptor B2 (LILRB2), which is expressed on dendritic cells, monocytes, and macrophages[59].

The cell-surface HC dimers do not seem to come from misfolding in the ER, but rather are thought to arise through endosomal recycling of conventionally folded B27[60]. The Cys67 residue is important for cell-surface homodimer expression.

In B27+ patients with SpA, KIR3DL2+ NK cells and CD4+ T cells are expanded in peripheral blood, compared with B27– SpA, rheumatoid arthritis, and other controls, and the NK cells from these patients showed a higher level of cytotoxicity[61]. These CD4+ T cells express more CCR6 and produce IL-17 and other cytokines. KIR3DL2+ has been thought to protect memory T cells from apoptosis, and Dr. Bowness's group have evidence for some protection of KIR3DL2+ NK cells from apoptosis by the B27 HC-dimer tetramers.

They hypothesize a mechanism whereby infection (or other innate immune stimuli) stimulates increased surface expression of B27 HC homodimers. Binding of B27 HC dimers to KIR and LILR could promote inflammation by

enhancing survival of NK and T cells and influencing differentiation of LILR-expressing antigen-presenting cells[62]. This hypothesis would still need to explain why surface homodimers are also seen with the subtypes poorly associated with AS[63], although the degree of homodimer formation by B*2709 was less than by B*2705. Dr. López de Castro commented that his group found HC homodimer formation in both B*1403 (associated with AS) and B*1402 (not associated with AS), although to a lesser degree than in B*2705. They have not looked at binding of these to any receptors. Both B*14 alleles show higher proportions of HC10-reactive (unfolded) surface expression than B*2705[64].

Dr. Sieper asked whether peptide specificity plays any role in these recognition phenomena. Dr. Bowness responded that since the inhibitory KIR3DL1 receptor shows pronounced peptide sensitivity in its interaction with conventionally folded B27[65], the B27 peptide repertoire could modulate the response to homodimers by cells expressing KIR3DL1, either alone or together with KIR3DL2.

Although rodents do not express KIR, they express LILR homologs, and Bowness's group has provided evidence that the B27 HC-dimer tetramers bind to these structures in B27 transgenic mice and rats[66].

The Role of Genes Other Than HLA-B27

Studies of monozygotic twins suggest that susceptibility to AS is more than 90% a matter of genetic inheritance, and B27 confers at most half of the genetic risk. There is evidence for a contribution from other HLA-B alleles (noted above) and other HLA loci, including HLA-DRB1 and DQB1, but not HLA.

Matthew Brown reviewed the extensive recent progress by the Australo-Anglo-American Spondylitis Consortium [TASC, headed by Drs. Brown, Reveille, and B. Paul Wordsworth (Oxford University)] identifying non-B27 genetic polymorphisms associated with AS. These recent results and a review article have just been published[63,67]. In the first genome-wide study of AS by the Wellcome Trust Case-Control Consortium (WTCCC) and TASC, single-nucleotide polymorphisms (SNP) linked to the genes ERAP1 (discussed below) and above-mentioned IL23R were found to be significantly associated with AS[50]. The new TASC study[67] employed over 288,000 SNP in surveying over 2000 patients with AS and almost 6000 controls, with a replication study of selected markers in another 947 patients and over 1500 controls.

Two newly identified loci with definite association to AS (defined as $p < 5 \times 10^8$, with replication) reside in regions with no identified genes, so-called intergenic regions or "gene deserts," one in chromosomal region 2p15 and the other in 21q22. The latter has been reportedly associated with pediatric-onset inflammatory bowel disease (IBD)[68], but the association with AS is seen even when patients with known IBD are excluded. Dr. Brown and his colleagues have isolated long noncoding RNA transcripts from each of these regions in order to investigate their significance, which is completely unknown. The other gene associations provisionally identified by TASC include IL1R2, which encodes a decoy receptor with high affinity for IL-1 and IL-1ß and low affinity for the IL-1 antagonist IL-1RA; and ANTXR2, which encodes the protein capillary morphogenesis protein 2 (CMP2), a transmembrane protein widely expressed primarily during capillary morphogenesis. Other genes for which recent publications support association with AS include TNFR1[63], a haplotype near TNFSF15[69], and a region near TRADD[70]. A search for associations with AS of 37 genes known to be associated with Crohn's disease showed evidence for association with a gene desert at 1p32 and with STAT3[71]. Interestingly, STAT3 is thought to be the master regulator for Th17 differentiation[72]. In his presentation, Dr. Brown also mentioned suggestive association with CARD9. He mentioned that the studies examining association of AS with the very complex polymorphism in the KIR region on chromosome 19q13.4 have not given consistent results, although positive associations have been reported[73]. He also described gene expression studies in PBMC from patients with active AS and matched controls, showing upregulation of the Th17 pathway and downregulation of the Th1 pathway.

The odds ratios (OR) for these genes are generally in the range of 1.1–1.5, as opposed to the OR for B27, which exceeds 100 in many populations. Low OR do not necessarily reflect biological unimportance, however, and it is reasonable to assume that a gene found associated by genome-wide screening is in fact involved in the pathogenesis of the disease[63]. These new genes provide powerful tools for gaining insight into the pathogenesis of AS and the role of B27.

The discussion brought out the point that B27 is the only AS gene so far that has been shown to be associated with age of onset, and that in general the genetic data do not correlate with known markers of disease severity or progression. Dr. Wordsworth emphasized the need for more detailed phenotyping as a key to interpreting the new genetic data.

ER Aminopeptidase 1 (ERAP1)

Nilabh Shastri (University of California, Berkeley, CA, USA) gave an overview of the function of ERAP1 in MHC class I peptide processing. This gene is encoded on chromosome 5p15. It has been identified by many other names, including ARTS1 (aminopeptidase regulator of TNFR1 shedding) and, in the mouse, ERAAP (ERA associated with antigen processing). In humans, it can form a heterodimer with the closely linked gene ERAP2, which shows no SNP association with AS. In mice, there is only one locus. Human ERAP1 and mouse ERAAP share 86% protein sequence identity, whereas ERAP1 and ERAP2 are only 50% homologous. ERAP1 and mouse ERAAP are ER-resident amino peptidases that are strongly upregulated by

IFN-γ. Peptides that are produced through the protease activity of the proteasome in the cytosol, transported by TAP into the ER, and bound to MHC-I molecules, are subject to amino terminal trimming by ERAP1[74]. ERAAP knockout mice show alteration in the MHC-bound peptide repertoire, with partial loss of MHC-I expression, binding of longer than normal peptides, and marked reciprocal CTL alloreactivity between ERAAP–/– and wild-type mice[74,75]. A dramatic example of the effect of loss of ERAAP is seen in H-2^d mice, in which protection against toxoplasmosis is dependent upon CTL recognition of a single peptide presented by L^d. This peptide is not generated in ERAAP–/– H-2^d mice, and these mice cannot respond to toxoplasma infection, but can be protected by prior immunization with the peptide.

Unlike IL23R and some of the other AS-associated genes, ERAP1 has not shown association with IBD or psoriasis. (It has shown association with cervical cancer, along with other genes associated with antigen processing, all thought to be related to the immune response to papilloma virus[76].) Since AS is almost unique in showing a near absolute genetic requirement for a particular MHC-I allele, this suggests that the obvious potential interaction between the MHC peptide editor ERAP1 and the B27 peptide repertoire forms the basis for the association of this gene with AS. However, the matter is not so simple. Stewart Levine and colleagues (NIH) have reported in a series of papers that the identical gene product that they have termed ARTS-1 functions indirectly to accomplish the ectopeptidase-mediated cleavage of several cytokine receptors, including TNFR1[77], IL-6Rα (CD126)[78], and IL-1R2[79]. Although Dr. Shastri's data indicate that ERAAP is expressed only in the ER, the data from the Levine group's experiments in completely different cell types indicate that ARTS-1 is an integral type II membrane protein that binds TNFR1 and forms part of a multiprotein complex[80,81]. Since cytokine signaling, and particularly TNF signaling, plays a central although as yet undefined role in AS pathogenesis, an effect on cytokine receptor shedding is quite a plausible role for ERAP1 involvement with AS.

Dr. Brown pointed out in his presentation that the ERAP1 SNP most closely associated with AS is very near the encoded catalytic site of the aminopeptidase activity. This supports the idea that peptide trimming in the ER is involved in AS pathogenesis and responsible for the genetic association. Drs. Robert Inman and Nigil Haroon (Toronto Western Hospital, Toronto, Canada) mentioned their findings that serum levels of the soluble forms of TNFR1, IL-1R2, and IL-6R in AS patients showed no correlation with ERAP1 or ERAP2 genotypes. Dr. Wordsworth's group is currently studying ERAP1 on several fronts, including solution of the crystal structure and functional analysis of several mutant variants.

It should be noted that Dr. Levine was unable to attend the meeting, and the discussion thus lacked his input.

HLA-B27 Transgenic Rats

Although B27 transgenic rats were not the topic of a single talk or session, they were an oft-recurring subject throughout the day's discussion. It has been 20 years since my colleague Robert Hammer (University of Texas Southwestern, Dallas, TX, USA) produced a series of rat lines transgenic for HLA-B27 and hß$_2$m, and we observed that the lines with high B27 transgenic copy number (≥ 40) developed a multisystem disease with features of SpA[41,42,82]. The principal features include colitis, gastritis, peripheral arthritis, rate epididymo-orchitis, psoriasiform skin lesions, and rare spondylitis.

As alluded to above, we sought to test Dr. Colbert's hypothesis that B27 HC misfolding induces the inflammatory disease. This was done by breeding in additional hß$_2$m transgenes, attempting thereby to rescue B27 HC from misfolding. This substantially decreased B27 HC misfolding and the attendant UPR, but surprisingly was associated with a dramatic increase in the frequency and severity of arthritis. Most dramatically, rats with 20 transgene copies of HLA-B27, which in the presence of 15 copies of hß$_2$m remain healthy, in the presence of 50 copies of hß$_2$m show a complete absence of GI inflammation, together with severe peripheral arthritis and tail spondylitis in a majority of the males, and epididymo-orchitis in all of the males. Rats with comparable transgene copy numbers of HLA-B27 and hß$_2$m remain healthy[58].

Several findings from the rat studies were discussed. It was recently reported that all of the disease features develop unimpeded in B27/hß$_2$m transgenic rats lacking a functional CD8a gene, despite profoundly impaired CTL responses[83]. Similar results have been observed in cell transfer and depletion experiments[84,85,86]. To the extent that the rat model mirrors the role of B27 in human SpA, these results challenge the hypothesis that CD8+ T cell recognition of B27 is central to disease pathogenesis. As emphasized by Dr. López de Castro, this by no means precludes a central role for the B27 peptide repertoire (peptidome), which influences many aspects of B27 biology[21,87], nor does it preclude a secondary role for CD8+ T cells.

Maxime Breban (Institute Cochin, Paris, France) and Balfour Sartor (University of North Carolina, Chapel Hill, NC, USA) described their findings of defective antigen-presenting cell (APC) function in the B27 transgenic rats. In Dr. Breban's experiments, splenic dendritic cells from the disease-prone transgenic lines, but not from the other transgenic lines, show impaired induction of proliferation of allogeneic CD4+ T cells, impaired immunologic synapse formation, impaired cytoskeletal function, and increased induction of Th17+ T cells (unpublished data and[88,89,90]). They recently observed similarly impaired stimulation of CD4+ T cells by dendritic cells from SpA patients, compared with healthy controls[91]. (It is interesting that a diminished mixed

lymphocyte reaction in patients with SpA was first reported over 30 years ago[92,93].)

In Dr. Sartor's experiments, intestinal APC (including B cells) from B27 transgenic rats showed less induction of CD4+ T cell IFN-γ production, but were less responsive to the IL-10- or transforming growth factor-ß-induced suppression, and splenic APC showed increased TNF production in response to TLR signaling[94,95,96]. The molecular basis for these phenomena remains to be identified but clearly must have something to do with the B27 and hß$_2$m transgenes.

Several investigators asked why such a high gene copy number is needed to see a disease phenotype in rats. Humans, of course, have only 2 copies of HLA-B alleles. There is conflicting evidence in the literature as to whether B27 homozygotes have increased frequency or severity of SpA. A recent large study of Finnish families suggested an increased frequency but no increased severity[97], which was consistent with a much earlier study by Dr. M. Asim Khan (Metro Health Medical Center, Cleveland, OH, USA)[98]. There has also been disagreement regarding levels of B27 expression in patients versus healthy controls. Dr. Brown stated that their gene expression studies (below) did not show evidence for increased HLA-B mRNA expression. A study from Dr. Sorrentino and her colleagues indicated significantly higher levels of B27 surface expression in PBMC from patients, but not overall higher levels of HLA class I nor of T cell activation markers or markers of disease activity or progression[99]. Dr. Breban's recent data support this finding[91].

We do not have comparative data on B27 expression between rats and humans, but the expression of the B27 and hß$_2$m transgenes at the rRNA and protein levels is copy number-dependent[58,82]. High copy hß$_2$m lowers the disease threshold of the B27 copy number and alters the disease phenotype, but rats with high copy hß$_2$m alone remain healthy[58].

It should be noted that, in rats, B27 HC and hß$_2$m are functioning in an environment in which all other factors are xenogenic, including the protein synthesis machinery, membranes, chaperones, peptide-loading complex proteins, and peptide repertoire, and in which there is competing rat MHC class I and ß$_2$m. Therefore, it may not be surprising if the key molecular processes by which B27 triggers disease operate under different kinetics than in humans. Moreover, the absolute timeframe for disease development is strikingly different in rats versus humans. The median age of AS symptom onset is 23 years in humans, whereas arthritis appears in rats at a median age of 150 days, a factor of 56. It is true that the entire biological program in rats is accelerated by a similar factor, compared with humans. Nonetheless if, as Dr. Colbert and others have suggested, the disease depends upon the accumulation of an abnormal protein product, overexpression of that product may be necessary in order for the rat disease to keep relative pace with the human disease.

It was proposed by Dr. López de Castro that the best way to clarify the disease-relatedness of the B27 subtypes and to have a uniform system for rigorously comparing their properties would be to make lines of transgenic rats, each expressing one of the subtypes, in particular, ones either strongly or weakly associated with AS. This approach presupposes the feasibility of making lines with the requisite transgene copy numbers of the B27 subtypes and hß$_2$m on the appropriate genetic backgrounds within reasonable limits of time and expense. It also presupposes that the other subtypes' behavior in rats would mirror the behavior in humans as faithfully as that of B*2705. If these assumptions are true, theoretically any of the subtypes, even the rarest, as well as other alleles such as B*1403, could be studied for disease susceptibility, and their molecular properties compared. There was agreement on the potential productiveness of this idea by many of the participants, but with some dissent.

The lack of a robust disease-associated B27 transgenic mouse model was noted[100], and it was suggested by Dr. Sartor and others that effort be made to identify a combination of genetic background and gene knockouts that might allow penetrance of a SpA phenotype in mice. It was also noted that technology now exists, and systematic efforts are now being made, to produce gene knockouts in rats[101]. These should add greatly to our understanding of the role of B27 in causing SpA in rats, and by extension, in humans.

Where in AS Pathogenesis Does B27 Act?

Irrespective of the actual molecular mechanism, it remains unresolved exactly what aspect of B27 pathogenesis is associated with B27. Dr. Sieper pointed out that the clinical feature showing the strongest association with B27 is axial inflammation in bone, cartilage, and enthesis. In his view, the role of B27 is in an immune response that initiates and/or perpetuates this inflammation. The severity of disease, degree of new bone formation, extent of peripheral arthritis, and extraarticular manifestations are largely due to factors other than B27. There was considerable discussion but general agreement with this formulation.

Dr. Hill Gaston (Cambridge University) pointed out that reactive arthritis overall is less associated with B27 than is its chronicity and progression to AS, suggesting that B27 may not necessarily be causing the most proximal events in pathogenesis. Dr. Inman mentioned their recent results from a Salmonella outbreak suggesting increased susceptibility to symptomatic infection associated with B27, while occurrence of acute post-Salmonella reactive arthritis was associated with TLR2[102,103].

Summary and Conclusions

The final session of the workshop was devoted to identify-

ing areas for future investigation. A suggestion was made to develop a form of Koch's postulates by which to test proposed mechanisms to explain the B27 AS association. Although this was not formally accomplished, Matthew Brown has recently published his own list for criteria for a proposed mechanism[63], suggesting that it should be:

1. Consistent with the subtype data
2. Consistent with the other known data about AS, including ERAP1 and IL23R associations
3. Supported by data from AS patients
4. Confirmed *in vivo* in an animal model

The first criterion presupposes that the subtype data are accurate. In addition to proposals to make B27 subtype transgenic rat lines and continuing to study populations, a number of other ideas were proposed. Dr. Sorrentino suggested better characterization of the HLA haplotypes, particularly for B*2706 and B*2704. Dr. Cresswell suggested sequencing entire HLA haplotypes in several individuals. Dr. Gaston suggested using B*2706 as the most weakly associated subtype, and extensively comparing its behavior to the known associated subtypes.

The second criterion would include continued investigation based on the newer genetic data. This would include continued investigation of the other associated genes and their interaction with B27. An approach mentioned by Dr. López de Castro was to study the behavior of B27 (his example was the peptide repertoire) in cells from asymptomatic individuals carrying as many of the disease-associated alleles as possible, and to compare them with those from B27 individuals with fewer of these alleles. Presumably, one would also follow such individuals prospectively.

The third criterion would need to explain the propensity for bone, cartilage, and enthesial inflammation, as emphasized at the workshop by Drs. Sieper, Inman, Tri Tran (New York University, New York, NY, USA), and others. This calls for continued progress in the immuno-osteology of AS. It might also be extended to include disease phenomena associated with B27 other than axial inflammation, at least including reactive arthritis and anterior uveitis. It would also include an explanation of the dramatic effect of anti-TNF therapy, as well as other pharmacologic effects, whether beneficial or not.

The fourth criterion includes further work in rats and mice, as described above. Dr. Shastri emphasized several times the importance of understanding the mechanism of disease as an approach to defining the role of B27, which applies to both human and animal studies.

Thirty years ago the late D. Bernard Amos described the discovery of the HLA system as "a page of nature read out of context"[104]. Applied to HLA, the association of HLA-B27 with AS would be described as a word out of context on that page out of context! Nonetheless, there is good reason to expect that, through the past and future efforts of those who attended this workshop, and those of many others, we will eventually see the whole context.

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