Citrullination and autoimmune disease: 8th Bertine Koperberg meeting

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Citrullination, the process in which citrulline-containing proteins are formed, seems to have a role in several autoimmune diseases. Although citrulline is a common metabolite present throughout the human body, it is a non-standard amino acid, which means that it cannot be incorporated into proteins during protein synthesis. Citrulline-containing proteins can only be generated through post-translational modification of arginine residues, a reaction that is catalysed by peptidylarginine deiminase (PAD) enzymes.

At the 8th Bertine Koperberg meeting in Nijmegen, The Netherlands, held in March 2005, the way in which citrullination might be involved in the autoimmune diseases rheumatoid arthritis (RA) and multiple sclerosis (MS) was discussed.

PAD ENZYMES, CITRULLINATION, AND ANTIBODIES TO CITRULLINATED PROTEINS

Ger Pruijn (Nijmegen, The Netherlands) gave an excellent overview of the enzymes responsible for citrullination. Five highly conserved isotypes of PAD enzymes exist in all mammalian species (PAD1-PAD4 and PAD6). The main difference between the isotypes is their tissue-specific expression. For RA and MS, PAD2 and PAD4 are the most interesting enzymes as both of them are expressed in the synovium and central nervous system (CNS). All PAD enzymes require high calcium levels for activity (about 10^{-5} mol/l). Because the intracellular calcium concentration in healthy cells is much lower (10^{-7} mol/l), the enzymes are only activated during events that lead to high calcium levels, like cell death, or late in the differentiation of skin cells (PAD1 and PAD3), as was explained by Michel Simon (Toulouse, France). Citrullination thus is believed to have an important role in several physiological processes, including the normal development of the myelin sheath and epidermis.

The effect of citrullination on protein function was also discussed. Whereas arginine is a positively charged amino acid, citrulline is uncharged, and citrullination of proteins thus leads to a loss of net positive charge. As a result, the target proteins adopt an altered, generally more open structure. This change in structure may severely hamper the cellular function of these proteins. Interestingly, all known natural substrates of PAD enzymes are proteins that have an important structural function (for example, intermediate filament proteins, myelin basic protein (MBP), histones, keratins, fibrin clots).

The question whether the presence of citrullinated antigens such as fibrin and vimentin is specific for RA was extensively discussed. Mireille Sebag (Toulouse, France) and Erik Vossenaar (Nijmegen, The Netherlands) presented data indicating that citrullinated proteins are generated during many different types of inflammation, and thus are not RA-specific. In contrast, Dominique Baeten (Ghent, Belgium) provided evidence that certain intracellular citrullinated proteins are generated only in RA. The precise nature of these RA-specific proteins, however, is still unclear.

Citrullinated proteins are also generated in arthritic animals and the citrullination was shown to increase arthritogenicity (Helena Erlandsson, Stockholm, Sweden). In this context it was interesting to hear the first preliminary results of studies with PAD knockout mice (Brian Wernegburg, Ridgefield, USA) and PAD overexpression (Han Zsendman, Nijmegen, The Netherlands).

CITRULLINATION AND MULTIPLE SCLEROSIS

The subject of citrullination and MS was introduced by Jon Laman (Rotterdam, The Netherlands) and George Harauz (Guelph, Canada). MS is a chronic inflammatory disorder of the CNS, characterised by destruction of the myelin sheath. The latter is formed by a cell layer that covers the axons of neurones. These cells, oligodendrocytes in the CNS, and Schwann cells in the peripheral nervous system, provide electrical insulation to the neurone, which is important for adequate conduction of nerve impulses. Demyelination causes loss of nerve signals, which in turn results in many different clinical manifestations (for example, loss of motor function, loss of vision, loss of sensation and paraesthesia).

The cells of the myelin sheath form a multilayer membrane structure around the axons consisting of lipid-protein layers. One of the major protein components is MBP, and this protein is known to undergo various post-translational modifications (phosphorylation, deamidation, arginine methylation, acetylation at its N-terminus, and also citrullination) (reviewed by Harauz et al.).

In healthy adult humans about 18% of the MBP molecules have six (out of 19) of their arginine residues converted to citrulline (MBP-cit6). The remaining MBP molecules do not contain citrulline. In patients with MS the proportion of MBP-cit6 is increased to 45% of total MBP. In addition, in the rare Marburg type of acute fulminating MS, 80% of the MBP molecules are heavily citrullinated (MBP-cit4).

The decreased net positive charge of MBP-cit6 weakens its interactions with the negatively charged phospholipids. Complexes containing MBP-cit6 are not as densely packed as those formed with non-citrullinated MBP. In addition, the partly unfolded structure of MBP-cit6 renders it more susceptible to proteolytic degradation, which might directly be related to the observed demyelination of axons in the brain of patients with MS. Reinout Raijmakers (Nijmegen, The Netherlands) and Anthony Nicholas (Birmingham, USA) used experimental autoimmune encephalomyelitis (EAE) mouse models for studying the process of citrullination in MS. They presented data showing that in these animals not only MBP but also other CNS proteins, such as the astrocyte-specified glial fibrillary acidic protein, are citrullinated. These data confirm the notion that citrullination of brain proteins is intimately associated with the demyelination process during the development of MS.

CITRULLINATION AND RHEUMATOID ARTHRITIS

Several speakers presented data on the antibodies to citrullinated proteins (ACPA) or cyclic citrullinated peptides (CCP) in patients with RA. After an overview of historical data (Guy Serre, Toulouse, France) and the clinical usefulness of these antibodies (Peter Schur, Boston, USA), additional new data were presented by a number of speakers. From these it can be concluded that anti-CCP antibodies and ACPA are extremely specific for RA (~98%) and present very early in the disease (Dörte Hamann and Ben Dijkmans, Amsterdam, The Netherlands; Björn Svensson, Lund, Sweden).
WHAT CAN WE EXPECT OF CITRULLINATION IN THE FUTURE?

William Robinson (Stanford, USA) has applied the microarray technique to profile autoantibody responses in cohorts of patients with RA and control patients. In addition to demonstrating that autoantibody targeting of citrullinated autoantigens is sensitive and specific for RA, patients with RA with ACPA responses frequently exhibit autoantibody reactivity against multiple citrullinated peptides and/or proteins contained on the arrays. He also presented data using a bead array to profile serum cytokines in RA, and observed raised levels of proinflammatory and immunomodulatory cytokines in about one third of patients with RA. Raised levels of serum cytokines were associated with presence of anti-citrulline autoantibody responses together with clinical features predictive of a more severe disease course.

The potential role of citrullination and anti-citrulline immune responses in the pathogenesis of RA was discussed extensively throughout the meeting. The data showing that anti-CCP antibodies can pre-date clinical RA by years in certain patients suggests that anti-citrulline responses may have a pathogenic role. The data presented demonstrating antibody activity to citrulline substituted fibrinogen peptides also supports this view. Although a substantial body of suggestive data exists, experimental data demonstrating a clear pathogenic role in autoimmune arthritis in mice or humans has not been presented to date. Adenoviral delivery of PAD4 to mouse joints did not evoke arthritis, and the PAD2 and PAD4 deficient mice did not exhibit clear alterations in the severity of EAE. Many investigators in the field are designing and performing experiments attempting to answer this question.

NEW THERAPEUTIC APPROACHES IN MS AND RA

Paul Utz (Stanford, USA) presented an overview of proteomic technologies to study RA and other autoimmune diseases. These technologies included mass spectrometry, antibody arrays, antigen arrays, lysate arrays, phosphoprotein flow cytometry, and other technologies. Utz also presented in depth development of “reverse phase lysate” protein array technology to study signal transduction in autoimmune diseases. Reverse phase lysate arrays are produced by printing lysates, generated from T or other immune cells over a time course after stimulation, in ordered arrays on nitrocellulose coated microscope slides. These arrays are probed with multiple distinct phosphoprotein-specific antibodies to profile kinase, phosphatase, and other signalling pathway molecule activation states. Finally, he presented an application of the use of this technology to analyse rare primary cell populations as shown in a study of differential STAT protein phosphorylation in interleukin 2 stimulated CD4+CD25+ regulatory T cells.

Although recombinant antibodies and soluble receptors enable specific blockade of cytokines and certain receptors, most of these treatments globally modulate immune function and are thereby not fundamental or curative. William Robinson (Stanford, USA) reviewed the development of antigen-specific tolerising DNA vaccines for the treatment of human autoimmune disease. Tolerising DNA vaccines deliver an autoantigen encoded in a mammalian expression vector by intramuscular injection. These vaccines have demonstrated efficacy in murine models of MS, autoimmune diabetes, and RA. Mouse model data demonstrate that such vaccines can induce specific anergy of the autoreactive T cells. They also reduce epitope spreading of autoreactive B cell responses.
SHOULD ANTI-CCP/ACPA POSITIVITY BECOME A CLASSIFICATION CRITERION FOR RA?
The meeting ended with a comprehensive discussion, led by David Pisetsky (Durham, USA), Josef Smolen (Vienna, Austria), and Peter Lipsky (Bethesda, USA), on the question of whether anti-CCP/ACPA positivity should become a classification criterion for RA. There was general agreement that because the anti-CCP/ACPA antibody system is more specific than, and at least as equally sensitive as, the rheumatoid factor, it could and probably should replace rheumatoid factor or be added to the existing classification criteria. However, because so many other new tools of possible relevance for the classification and diagnosis of RA (for instance in the field of imaging and genetics) have become available recently, the majority favoured that such additions should be part of a comprehensive revision of the 1987 ACR criteria. The ACR and EULAR are already discussing this topic.

The organisers wish to thank the following sponsors: Abbott, Axis-Shield, EuroDiagnostics, Medical and Biological Labs Co Ltd, MSD, Nationaal Reumafonds, Organon, Pfizer, Pharmacia Diagnostica Roche, Schering Plough, Wyeth, Landelijk Katholiek Reumacentrum.

The ASTIS protocol, consisting of high dose cyclophosphamide and bone marrow transplantation (EBMT), was launched in 2004 under the auspices of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation International Scleroderma (EBMT) to compare safety and efficacy of high dose immuno-suppressive treatment (HDIT) and autologous haematopoietic stem cell transplantation (HSCT) with 12 monthly treatments with intravenous pulse cyclophosphamide (750 mg/m²). The trial builds upon the results from previous pilot studies conducted in various European centres, which demonstrated the feasibility and potential long term benefits of HDIT+HSCT in patients with severe scleroderma autoimmune diseases, including progressive systemic sclerosis. To combine safety and efficacy, a medium intensity regimen was chosen to achieve immunoaolation in the ASTIS protocol, consisting of high dose cyclophosphamide (2 × 2 g/m²) + Filgrastim (granulocyte-colony stimulating factor; 10 µg/kg) for mobilisation of haematopoietic stem cells, leukapheresis, and CD34 selection of the apheresis product, conditioning with high dose cyclophosphamide (200 mg/kg, rabbit ATG 7.5 mg/kg), followed by HSCT.

The ASTIS trial targets patients with early diffuse systemic sclerosis at risk of early mortality. These include patients with disease duration of ≤ 4 years, a modified Rodnan skin score (mRSS) of at least 15 (out of a maximum of 51), evidence of heart, lung, or kidney disease; and patients with a maximum disease duration of 2 years, an mRSS of 20 or more plus laboratory signs of acute phase reaction. Exclusion criteria are end stage organ failure, extensive pretreatment with cyclophosphamide (over 5 g intravenously or 3 months' oral treatment), treatment related mortality or unexpected toxicity has been seen in either treatment arm (median follow up 24 months, range 1–51). EBMT registered transplant centres with expertise in the treatment of severe scleroderma are highly encouraged to participate in this unique joint effort, which will remain open until 2008. Information on the trial is regularly updated on the trial’s website: http://www.astistrial.com (accessed 15 August 2005). It is hoped that the ASTIS trial, and its North American counterpart (the SCOT trial) will result in more effective treatment of patients with severe systemic sclerosis, with the aim of inducing a durable remission in the majority of patients treated.

ACKNOWLEDGEMENTS
The ASTIS trial is supported by grants from Amgen Europe (unrestricted educational grant), the Horton Foundation in Switzerland, the Groupe Francais de Recherche sur la Sclerodermie, and the Direction de la Recherche Clinique Assistance Pubbliche Hopitaux de Paris in France, and EULAR.

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REFERENCES


Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial: hope on the horizon for patients with severe systemic sclerosis

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