Available online http://arthritis-research.com/content/3/1/034

Meeting report

Sixth International Workshop on Scleroderma Research, Oxford, UK, 30 July–2 August 2000

Frank A Wollheim*, Christopher P Denton† and David J Abraham†

*Department of Rheumatology, Lund University Hospital, S-221 85 Lund, Sweden
†Centre for Rheumatology, Royal Free and University College Medical School, London, UK

Correspondence: Frank A Wollheim, MD, Department of Rheumatology, Lund University Hospital, S-221 85 Lund, Sweden; Tel +46 46 172280; fax +46 46 128468; e-mail Frank.Wollheim@reum.lu.se

Received: 16 August 2000
Revisions requested: 10 October 2000
Revisions received: 13 October 2000
Accepted: 18 October 2000
Published: 6 November 2000

Arthritis Res 2001, **3**:34–40

(Print ISSN 1465-9905; Online ISSN 1465-9913)

Abstract

We discuss major topics presented at this recent international workshop, illustrating how recent progress in areas as diverse as free radical biochemistry, developmental biology, molecular genetics and vascular biology is facilitating greater understanding of the multisystem connective tissue disease scleroderma. Some of the opportunities for translating this into novel and improved therapy are considered.

Keywords: experimental, human, organ manifestations, pathogenesis, scleroderma, therapy

Introduction

Some 200 scientists and clinicians gathered at Keble College in Oxford, UK from 30th July to 2nd August for this biennial meeting, which has grown immensely both in size and quality of research presentations. The college, built in the holy zebra style (1870), and the adjacent famous university natural history museum formed a most charming environment for the meeting. The conference was organized and co-chaired by Carol M Black (Royal Free Hospital, London, UK) and Joseph H Korn (Boston University School of Medicine, Boston, MA, USA). Plans are to arrange the next meeting in the Boston area in the summer of 2002.

Hunting for pathological genes in scleroderma

Fiona Brew (Cambridge, UK) presented the Affymetrix Gene Chip technology, which is a powerful method allowing analysis of a large number of gene expressions from single cells. It was presented as versatile, having high sensitivity and low risk for false-positive results. It is best suited for classification of single-cell diseases such as leukemias or other malignancies, and has found clinical application in distinguishing between acute lymphoblastic and myeloid leukaemia. The price is high, however, and as Constantin Bona pointed out in the discussion there are several causes for negative results. To this Dr Brew responded that one needed only 3–5 gene copies expressed per cell.

Next, David Strehlow (Boston, MA, USA) presented exciting data regarding a new genetic marker relating to scleroderma. This work utilized in parts the cDNA microarray technique above. As demonstrated by *in situ* hybridisation, the gene encoding the protease nexin 1 (*PN1*) is expressed only in scleroderma skin. Comparing cultured fibroblasts from normal, lesional, and nonlesional scleroderma skin, it was found that mRNA as well as protein

ACE = angiotension-converting enzyme; HSP = heat-shock protein; IL = interleukin; NO• = nitric oxide; TGF = transforming growth factor; TSK = tight skin.

secretion was 3–5 times higher in scleroderma. A search for other overexpressed genes by the microarray chip technique revealed overexpression of heat shock protein 90 (HSP90), a chaperon found in complex with other HSPs. HSP90 is essential for steroid hormone signalling. Expression was higher in lesional than in nonlesional skin. In cultured fibroblasts collagen transcription was doubled 3 h after exposure to HSP90. The effect lasted up to 24 h. Geldanomycin is known to inhibit HSP90, and it was shown that geldanomycin inhibited both collagen synthesis and the known effect of transforming growth factor β, TGF-β, on collagen synthesis. This prompted investigation of the mechanism for this inhibition. Geldanomycin was shown to inhibit the movement of SMADs from the cytoplasm to the nucleus. SMADs are proteins that are essential for the transmission of extracellular signals from TGF-β superfamily peptides via cell surface receptors to the nucleus. Furthermore it could be shown that HSP90 binds to SMAD3 but not to SMAD4. The biologic implication of SMAD3 binding may be that its attachment to the cell surface TGF-β receptors is broken. SMAD3 can then bind to SMAD4, and the complex can be transported to the nucleus and there influence promoter regions of certain genes. In essence the work suggests a new disturbed signalling pathway in scleroderma with profound effects on nuclear gene regulation of components of the extracellular matrix, such as collagen. In the extension this could lead to new therapeutic approaches. The designation of SMAD proteins comes from homologies with a *Drosophila* protein family called MAD and the *C. elegans* homologue SMA. The interested reader is referred to two recent reviews on the subject [1,2].

Timothy Wright (Pittsburgh, PA, USA) presented his work on gene expression in lesional and nonlesional skin cultured fibroblasts. The approach was to utilize a technique called serial analysis of gene expression, SAGE, allowing simultaneous display of 9–13 base-pair tags. The results were still incomplete, but indicated several differences between unaffected and affected skin fibroblasts. It was, however, pointed out in the discussion that even unaffected scleroderma skin is not normal. The technique used, although very powerful, did not pick up HSP90 overexpression.

Lance Fors (Madison, WI, USA) presented another analytic technique called Cleavase Fragment Length Polymorphism (CFLP), which is the flagship of the company at which he is chief executive officer. It is based on conformation rather than sequence specific Cleavase® enzymes, which are thermostable and structure-specific. They recognize and cleave the junctions between single-stranded and double-stranded DNA at the 5′ side. The newly formed fragments fold in a characteristic way and their analysis gives information on single nucleotide polymorphisms, SNP. SNPs are the most common genetic variations among individuals, occurring at an estimated frequency of at least 1% in the population and affecting approximately 1 in 1000 nucleotides or as many as a million bases per genome. CFLP has the advantage over PCR in being faster and easier to use, since it does not involve gel electrophoresis and eliminates the risk of carry-over contaminants. It has been successfully applied to routine detection of coagulation factor V mutation (Leiden), which predisposes to thrombosis. This technique promises to come into wide use for detection of genetic diseases and gene expression. What it will bring for scleroderma research remains to be seen.

Animal models and the pathogenesis of scleroderma

Sergio Jimenez (Philadelpia, PA, USA) reviewed the field of experimentally induced and spontaneous animal models of fibrosis, concentrating on the spontaneous tight skin mouse (TSK1) model. This model was first found in 1976, and is linked to a duplication of parts of the fibrillin 1 gene, which leads to overproduction of collagen in the skin, heart and other internal organs. In its homozygous form it is lethal. A similar abnormality has been described in humans by Stephanie Jablonska of Warsaw, Poland and called congenital facial dystrophy. Dr Jimenez, who worked for years elucidating the TSK abnormality, has (in my mind) unjustly been accused of studying an irrelevant model. The gene duplication is 40 kb long, which is rather unusual. It has two TGF-β-binding sites and one extra RGD-binding site. This explains how the increased collagen production comes about. New experiments have shown that the TSK1 model is not dependent on T cell function, which is one limitation of this model.

Jose Pablos (Madrid, Spain) had studied the TSK skin in the neonatal period from the first to the 35th week. Both normal and TSK mice had more proliferation and apoptosis only in the newborn state and not later. Thus neither defect (apoptosis and increased proliferation) was involved in the TSK abnormality, which is characterized by a continued high rate of collagen and matrix synthesis in neonates rather than by acquisition of new genes, according to Pablos.

Constantin Bona (New York, NY, USA), who had shown previously that the defective fibrillin-1 gene in TSK mice had indeed a higher binding capacity for TGF-β, had now studied TSK offspring with interleukin (IL)-4R or TGF-β mutated genes. IL-4R$^{-/-}$ animals showed a normal phenotype, whereas heterozygous animals were not protected. TGF-β$^{-/-}$ was lethal and heterozygous animals had the TSK phenotype. No mutation affected lung fibrosis, which thus may have a different pathogenesis. He also indicated that mice transgenic with the mutant fibrillin gene demonstrate thicker skin but no lung disease, raising questions about tissue-specific expression of the transgene.

Stephen Clark (Farmington, CT, USA) reported studies on collagen gene expression in TSK mice. Only a subset of fibroblasts is activated, and remains activated, in culture. Collagen 1α1 was almost three times more highly expressed in these cells and TGF-β receptor 1 (TGF-βR1) was also upregulated, but to a lesser degree. Taken together all the information supports the hypothesis that fibrillin–TGF-β interactions are important for persisting fibroblast activation *in vivo*. It remains to be shown how relevant this is for understanding scleroderma in humans. Several transgenic approaches are in progress, some using the elegant gene switch technique involving *Cre* lox guided gene expression, which is a refinement of the knockout technique and allows the turning off of selected genes in certain tissues at precise time points only. Christopher Denton (London, UK) reported progress in studies of mice transgenic for TSK. He has identified enhancer elements in the far upstream region of the col1α2 gene in TSK mice. These elements were expressed in transgenic animals and correlated to fibrosis. Interestingly, the altered gene seems specific for fibroblasts, and does not affect collagen synthesis by osteoblasts.

Humphrey Gardener (La Jolla, CA, USA), working with integrins, had previously shown that fibroblasts from integrin α1 null mice lack feedback inhibition of collagen production when grown in collagen gels. He now presented new data indicating that α1 integrins prolong collagen promoter activity. Interestingly, mice with α1β1 integrin mutation had normal skin, despite increased hydroxyproline incorporation, indicating increased collagen synthesis. However this could be explained by a concomitant increased expression of some collagenases. If mice with the α1 deletion are crossed with collagenase resistant mice, a thick skin phenotype is expressed.

The α1β1 integrin was originally called very late antigen-1, VLA-1, on the basis of its expression on activated T lymphocytes. Philip Gotwals (Cambridge, MA, USA) pointed out the upregulation of this integrin in certain models of inflammation, such as *Mycobacterium butirricum* mineral oil arthritis. A monoclonal antibody to VLA-1/α1β1 integrin inhibits edema and inflammation in these animals. VLA-1 is only expressed on tissue cells. It may be involved in some forms of renal disease, and is a perhaps a putative target for intervention in scleroderma.

TGFβ biology and signalling

Anita Roberts (Bethesda, MA, USA) was the keynote speaker in this session. She pointed out that of the three forms of TGFβ, TGFβ1 is most important. The TGFs activate specific cell-surface receptors and these, upon activation, act as serine-threonine kinases. This starts the above-mentioned SMAD pathway that eventually leads to nuclear gene activation. There are at least nine different SMADs; some are activators, one is only a transporter (SMAD4), and two (SMADs 6 and 7) are inhibitors. SMAD7 is activated by IFNγ. To study the biology further, gene disruption experiments had been performed, which showed that all total deletions were lethal with the exception of SMAD3. SMAD3$^{-/-}$ animals show progressive wasting and mucosal infections as well as abscesses. TGFβ injection into wild-type animals causes mobilization of inflammatory cells, but this does not happen in the SMAD3$^{-/-}$ animals. Lack of chemotaxis and accelerated early phases of wound healing was another feature of the mutated animals. Furthermore, re-epithelialization was delayed both in homozygous and heterozygous animals, as was the normal auto-induction of TGFβ. The role of TGFβ in bleomycin-induced fibrosis and in early phases of radiation fibrosis was also emphasized. Interestingly, SMAD3$^{-/-}$ mice were resistant to radiation.

John Varga (Chicago, IL, USA) further explored SMAD biology in an effort to understand their regulatory functions in cell biology. He took the difficult approach by studying regulation in normal rather than in transformed cells. Normal adult skin fibroblasts as well as foreskin fibroblasts express both SMAD3 and SMAD4 after stimulation with TGFβ, and transport of these mediators to the nucleus takes place. A surprising finding was that mRNA for SMAD3 diminished after TGFβ administration. TGFβ also enhances SMAD7, which is an inhibiting SMAD. This indicates an autoregulation of SMAD activity and may explain some conflicting results regarding the influence of TGFβ on collagen synthesis *in vivo*. It is known that IFNγ abrogates TGFβ signaling. This effect is SMAD-mediated. Thus TGFβ not only triggers SMAD activation when ligating its cell membrane receptor, but also has a profound effect on SMAD expression and intracellular trafficking. A short general review on the regulation of SMAD activity was published by Wrana [1].

Sara Dallas (Manchester, UK) presented her work on latent TGFβ and its binding protein, latent TGFβ-binding protein or LTBP1. TGFβ proteins are secreted in a latent form of approximately 100 kDa, which binds to LTBP1 with a size of about 190 kDa. LTBP1 belongs to the fibrillin superfamily and is also a structural extracellular matrix protein that binds calcium. LTBP1 has mostly been studied in relation to bone and cartilage, but its relation to fibrillin and binding of TGFβ makes it a possible target for intervention in scleroderma.

The final speaker in this session, Ante Jelaska (Boston MA, USA), dealt with TGFβ and apoptosis. TGFβ induces a cell called a myofibroblast, expressing both the muscle cell marker alpha smooth muscle antigen and collagen. These cells are removed by apoptosis. Examining normal and scleroderma skin, it was found that 30% of normal fibroblasts were “apoptosis resistant”, whereas only 5% of lesional fibroblasts in culture showed this resistance. Scleroderma

fibroblasts thus are more prone to apoptosis. TGFβ reduces apoptosis.

TGFβ as a target for therapy

Stephen Ledbetter (Framingham, MA, USA) stated three principle methods for inhibition: antibodies, soluble receptors and low molecular weight compounds. He focused on a monoclonal antibody named 1D11. This is a high-affinity antibody which neutralizes all three TGFβ forms, 1, 2 and 3, and has a half-life of 15 h in the rat. In a genetic rat model of hypertension and renal fibrosis, 1D11 reduced elevated levels of TGFβ1 and TGFβ2, reduced proteinuria, and reduced expression of the alpha smooth muscle antigen and collagen synthesis in fibroblast cultures taken from renal biopsies. 1D11 also reduced blood pressure and increased renal medullar blood flow. However, disappointingly, it did not increase glomerular filtration rate in these animals. Another model under study was the unilateral renal artery obstruction, also in rats. These have in increased production of collagen type III mRNA and increased apoptosis. 1D11 normalized both abnormalities. The third model was subtotal (5/6) nephrectomy. 1D11 improved survival as do angiotension-converting enzyme inhibitors in this model. The antibody also inhibits collagen overproduction in experimental bleomycin lung fibrosis. This highly interesting antibody was not yet humanized, but this was in planning.

Anita Gilliam (Cleveland, OH, USA) studied a mouse model of scleroderma-like graft versus host disease, Scl GVHD, which is distinct from the more common lupus like GVHD. The scleroderma-like features include skin and lung fibrosis, increased synthesis of collagen, and TGFβ1 mRNA. Interestingly, these models are based on producing chimeric animals in which bone marrow and spleen cells from B10.D2 mice are transplanted into lethally irradiated BALB/c mice across minor histocompatibility loci, leading to a sclerodermatous graft-versus-host disease. The skin thickness in such animals is increased by 50% and this can be monitored *in vivo* with ultrasound. The animals do not produce autoantibodies, but skin infiltration by Mac-1-positive macrophages occurs in later stages. Increased production of TGFβ occurs earlier according to *in situ* hybridization analysis. It is not clear if lymphocytes play any role, but large histiocytes and mast cells can be seen and studied in digests from skin. These are CD11b positive and are the predominating cell type by day 21. They are probably chimeric cells. A polyclonal antibody prepared in Dr Gilliam's laboratory, when administered on day 1 and day 6, was able to prevent both skin and lung fibrosis and prevent skin infiltration with CD11b positive cells. It was not clear whether established skin fibrosis could be reversed.

David Glover (Cambridge, UK) reported on interesting early human trials of two anti- TGFβ2 human IgG4 antibodies, called CAT 152 and CAT 192, selected by phage antibody display technology. After promising results in animals, CAT 152 was tried in glaucoma drainage surgery. This study was stopped after the observation of an unusual form of abnormal sheeting around retinal arteries in three patients. Another study with this antibody in prevention of fibrosis after glaucoma surgery was positive, however, and superior to current routine therapy. The other antibody, CAT 192, is in phase I study for safety. The potential interest to use these or similar agents in scleroderma patients is obvious.

Vasculopathy and its control

James Seibold (New Brunswick, NJ, USA) started didactically, by pointing out differences between fibrotic irreversible, inflammatory, and vascular abnormalities in scleroderma. The latter consist of, for example, nephropathy, pulmonary hypertension, sudden cardiac death, and visceral and peripheral Raynaud's. He also pointed out the need for better biological markers.

Ariane Herrick (Manchester, UK) talked about oxidative stress and attempts to control it. Free radicals cause lipid peroxidation. The main actors are hydroxyl ($HO^{\bullet}$), superoxide ($O^{\bullet}$) produced during phagocytosis and reperfusion, and nitric oxide ($NO^{\bullet}$) released from vascular endothelium. Environmental toxins are a further source of oxidative stress. Reduced thiol levels, increased 9,11-linoleic acid levels, reduced ascorbic acid and selenium levels all support the occurrence of oxidative stress in disease. Low-density lipoprotein isolated from patients with scleroderma was more susceptible to oxidation *in vitro*. Although much evidence is in support, therapeutic trials with superoxide dismutase (SOD) or with a combination of antioxidant nutritional supplements combined with allopurinol have failed to show any benefit.

Jill Belch (Dundee, UK) talked about therapy with $NO^{\bullet}$ donors. She pointed out that, although not mortal, Raynaud's contributes significantly to morbidity in scleroderma. Scleroderma patients show diminished vasodilatation upon exposure to acetylcholine but normal response to nitroprusside administered by iontophoresis. However oral administration of the $NO^{\bullet}$ donor, L-arginine, had no significant effect on response to acetylcholine or nitroprusside.

Lily Shen (Ann Arbor, MI, USA) addressed endothelin antagonists. This is a large field of current development. Animal studies showed that, in animals exposed to hypoxia, ET-1 levels rise and pulmonary arterial pressure goes up. An ET-1 antagonist inhibited this rise in pulmonary arterial pressure. Although endothelin is a well-documented vasoconstrictive factor in scleroderma, and a number of inhibitors are available, no substantial practical results have been reported to date.

Prostanoids and pulmonary hypertension

The use of stable prostaglandin I preparations in primary pulmonary hypertension, pulmonary hypertension secondary to scleroderma and other circulatory disturbances is a success story which started more than 10 years ago. Glyn Belcher (London, UK) presented an overview without too many new aspects. The problems of high cost and intravenous administration limit the use of this therapy. Studies are in progress with oral and inhalation preparations, which may in part overcome this problem.

Thomas Medsger (Pittsburgh, PA, USA) presented a retrospective study based on more than 2500 patients observed for more than 11 years on average, and which was started by Dr Virginia Steen, now in Washington, DC, USA. Comparing patients with pulmonary hypertension and pulmonary fibrosis, the outstanding clinical difference was the rapid progression of dyspnea after onset in the former. Of the 182 cases identified, 80% were women, and 145 had the limited form of scleroderma. The mean disease duration when symptoms started was 13 years. Risk factors were difficult to identify, although 97% had Raynaud's compared to 96% of controls. Past use of calcium channel blocker was more common, as was prevalence of skin ulceration, severe Raynaud's, and occurrence of new ulcers coinciding with onset of dyspnea. Pulmonary function tests showed forced vital capacity to be 78 ± 18% compared to 67 ± 20% in 393 patients with interstitial lung fibrosis and 87 ± 20% in patients without evidence of lung involvement. All figures are expressed as percentage of the expected normal. The corresponding figures for transfer factor for carbon monoxide were 43%, 54% and 76%. Anticentromere antibodies were found in 65% but anti-topoisomerase antibodies in only 2% of the patients. The corresponding figures for control patients were 34% and 37%. Eleven patients had a history of scleroderma renal crisis on average 4 years before onset of dyspnea. Of these only one patient was still alive under continous Flolan therapy. Four patients had undergone lung transplants; two survived. In order to be considered for transplantation patients had to be nonsmokers, under the age of 65 and without evidence of aspiration. Monitoring of pulmonary pressure by Doppler echo under stress was probably better than resting Doppler.

Lewis Rubin (La Jolla, CA, USA) talked about the pathogenesis of pulmonary hypertension. He started by pointing out the remarkable diversity of conditions that respond to prostacyclin therapy. These include intrinsic vascular disease, primary pulmonary hypertension, scleroderma- and lupus-related pulmonary hypertension, Eisenmenger's syndrome, thromboembolic conditions etc. These do not respond to calcium channel blocking agents. Thus it seems unlikely that vasodilation is the mechanism of action. Chronic effects include vasodilation, but also anti-platelet and anti-proliferative effects. The histology of pulmonary hypertension involves marked thickening of all three layers of the vessel wall. The surprising fact is that these changes are reversible. One can distinguish three stages: a preclinical stage with rising pulmonary pressure; a symptomatic stage with relatively stable pressure and preserved cardiac output; and a progressive declining stage with falling cardiac output. Left ventricular function is decisive for survival. Potential mediators could be increased levels of 5HT or thromboxane, increased endothelin or reduced endothelin resistance. The ratio of thromboxane to prostacyclin may be one important factor. Increased endothelin expression is another factor in the progressive stage. Closed K^+ channels are also a pathogenic factor. The most important acute effect of prostacyclin therapy is to improve cardiac output rather than to reduce pulmonary pressure. Withdrawal of prostacyclin may lead to life threatening rebound. In future work, it will be important to study the mechanism of prostacyclin effect, and also to try NO, endothelin receptor antagonists, selective phosphodiesterase inhibitors, K^+ channel openers, and perhaps gene therapy.

Fibroblasts, myofibroblasts and fibrocytes

Thomas Krieg (Cologne, Germany) summarized some of the enigmatic facts regarding the fibroblast, of which he is an eminent scholar. Our ignorance was attributed to two factors. There is no reliable marker for fibroblasts, and fibroblasts have not received enough research attention. Fibroblasts are multifunctional and occur in different shapes in inflammatory tissue, in the blood stream and in bone marrow. As shown by his countryman Bayreuther, normal fibroblasts develop from stem cells and occur in three different forms: mitotic, postmitotic and degrading. Seven different stages may be distinguished. The switch from mitotic to postmitotic fibroblasts can arise spontaneously or be induced by treatment with mitomycin C. Collagen synthesis is low in early fibroblasts and increases as the cells change into later stages of development. Mechanical tension, cytokine or integrin or other environmental stimulation can effect activation of resting fibroblasts, and can manifest itself transiently or permanently or result in transformation into the myofibroblast form. Clonal selection occurs in tissues as a result various stimuli, which include cell to cell interactions, and may, as in the case of scleroderma, result in excessive extracellular matrix formation. All this information is important to keep in mind when assessing the relevance of *in vitro* results with cultured fibroblasts.

Richard Bucala (Manhasset, NY, USA) talked on his favorite subject, the fibrocyte, a cell he has characterized in peripheral blood. This has distinct surface markers, collagen/CD13/CD34/CD45, and is probably a precursor cell for tissue fibroblasts. Previous animal experiments had shown that fibrocytes can present antigen. Bucala

reported on an experimental wound model, where an inserted sponge allowed fluid collection in order to study the repair process. Within a day, spindle-shaped cells invaded the sponge. Although these produced collagen and were CD34-positive, and had MHC as well as adhesion molecules expressed on the surface, they lacked monocyte markers. Bucala reminded us that Metchnikov had predicted the existence of such cells. Fibrocytes were also shown to carry chemokine receptors. They have been implicated in infections such as borreliosis and schistosomiasis, and clearly are important in wound healing. It is not defined where in the cell hierarchy they belong, it is not unlikely that they are precursors of the myofibroblasts. Engineered fibrocytes could become of interest as antitumor agents.

Three talks dealt with different regulating mechanisms of fibroblast function. Livingston Van der Water (Boston, MA, USA) described his work with fibronectin gene transcripts resulting from alternative splicing events which occur during development, tumor growth and tissue repair. This results in fibronectin epitopes in the so-called EIIIA and EIIIB regions, which transfer messages to fibroblasts. Boris Hinz (Geneva, Switzerland) dealt with the myofibroblast, which is distinguished by its expression of alpha smooth muscle actin, alphaSMA. This is brought about by TGFβ in concert with ED-A fibronectin, and functional SMA is needed for wound contraction. Direct proof of this was presented. Blocking of the N-terminal decapeptide of alphaSMA had previously been shown to abolish collagen contraction and polymerisation. The actual binding structure has now been identified as AcEEED (Acetyl-glutamic acid–glutamic acid–glutamic acid–aspartic acid). Blocking of this structure *in vivo* abrogates wound contraction. It is not known if myofibroblasts can be dedifferentiated. Veli-Matti Kahari (Turku, Finland) studies the regulation of collagenases and collagen synthesis. Matrix metalloproteinase (MMP)13 or collagenase III plays an important role in repair, as shown in an experimental model of gingival ulceration. MMP13 mRNA is induced by extracellular signals including TNFα and TGFβ. This is effected through the extracellular signal-regulated kinase-1/2 and the p38MAPK (mitogen-activated protein kinase) pathway. This also leads to diminished collagen synthesis and reminds us of the Bayreuther model mentioned above.

More on the vascular endothelium and oxydative stress

Joseph Loscalzo (Boston, MA, USA) reported on progress in our knowledge regarding the important functions of $NO^{\bullet}$ in regulating vascular tone and preventing thrombosis. $NO^{\bullet}$ inhibits thrombin receptor-activating peptide-induced phosphoinositide 3-kinase activity in human platelets, and this probably explains the de-aggregating effect of $NO^{\bullet}$. Intracellular cGMP increase and calcium reduction are involved in the reduced adhesion. A family with genetic platelet unresponsiveness to $NO^{\bullet}$ was studied, and two children of 4 and 7 years old already showed signs of vascular thrombosis. The roles of $NO^{\bullet}$ and oxidative stress in critical illness were the topics of a talk by Tim Evans (London, UK). There is evidence from studies of bronchoalveolar lavage fluid that neutrophils are implicated in nitration and hydroxylation damage to proteins. Similar mechanisms could operate in the anoxia in scleroderma. Augustine Choi (New Haven, CT, USA) reported on heme oxidases HO-1 and HO-2. These enzymes produce CO. HO-1 is induced by oxidative stress and is a protective mechanism; the protection is mediated by CO. CO, like $NO^{\bullet}$, activates the enzyme guanylyl cyclase, leading to formation of cGMP.

Cardiac and pulmonary fibrosis

Gerry Coghlan (London, UK) introduced the subject by pointing out that asymptomatic fibrosis is not uncommon, as shown in postmortem studies. Karl Weber (Memphis, TN, USA) then gave an exciting expose of his work on the fibrotic aspects of myocardial infarction. His main message was that scar formation after a myocardial infarction is a dynamic process, and that fibrosis is not confined to the site of initial damage but occurs in the entire circumference of the heart. He worked in an animal model with coronary artery ligation. Interestingly this fibrosis could be ameliorated by administration of ACE inhibitors or angiotensin receptor 1 antagonists like Losartan. Ron du Bois (London, UK) gave an overview of the distinct features of scleroderma-related pulmonary fibrosis, and pointed to the still not fully explored utility of high-resolution computer tomography, HRCT. The treatment indications were also discussed, and it was clear that early treatment in asymptomatic stages is a field of debate. The role of BAL findings is also controversial, although advocates say that the quantity of neutrophils but not that of eosinophils is helpful. It can be concluded that there is still room for better diagnostic and prognostic information. Barbara White (Baltimore, MD, USA) presented experience of treating fibrosing alveolitis with cyclophosphamide. Of 39 patients treated, 28 had a good outcome; among 30 not treated, only 7 had a good outcome. These figures can be compared with 34 patients with no evidence of alveolitis, of whom 27 had a good outcome. My conclusion is that this uncontrolled study, despite its limitations, is another piece of evidence for the efficacy of cyclophosphamide in this group of patients.

Therapeutic approaches to scleroderma

Dan Furst (Seattle, WA, USA), set the scene in this clinical session by giving a clear overview of the state of the art regarding clinical trials in scleroderma. The difficulties often encountered include selecting valid end-points and recruiting sufficient patients for reliable assessment. Novel approaches to therapy included immunoablation with autologous peripheral stem cell rescue, discussed by Alan Tyndall (Basel, Switzerland); this approach is currently

being evaluated in the USA and Europe using similar and well-controlled study protocols. Hermann Waldmann (Oxford, UK) discussed the potential for using antibody therapy directed against activated T cells, and in contrast Marc Feldmann (London, UK) presented an overview of the scientific background and trial results which form the rational basis for remarkably successful use of TNFα neutralizing treatments for rheumatoid arthritis. Overall, these presentations left the impression that, once pivotal mediators or cell types for scleroderma are identified, then the principles of targeted therapy are already validated. Translation of basic scientific observations into the clinical arena is now feasible and this is reflected by the smooth integration of clinical and basic research topics at the workshop.

Licensing of new drugs for scleroderma

This lively session considered the particular difficulties with obtaining licensing approval for novel therapies for scleroderma. Despite many good trials and a number of promising agents there are currently no approved disease-modifying treatments for scleroderma. R Shah (London, UK) presented a detailed account of the procedures needed to license a drug through the Medicines Controls Agency in the UK and James Seigal (Rockville, MD, USA) gave an illuminating overview of Food and Drug Administration (FDA) approaches, including some of the schemes which are specifically targeted towards rare diseases such as scleroderma.

Concluding comments

We hope it is evident from this account that scleroderma research is moving forward in several ways. New therapeutic approaches are obviously arising from the advances in molecular biology. An example is the recently published study of recombinant human Relaxin [3], which, however, did not show striking benefit and also showed an unexplained lack of dose response. Mortality in scleroderma remains higher than in other connective tissue disorders. This field needs the dedicated concerted actions and communication facilitated by international workshops.

Acknowledgements

Educational grants to support this conference were provided by The Scleroderma Foundation (USA), The Scleroderma Clinical Trials Consortium (USA), The Wellcome Trust (UK) and The Arthritis Research Campaign (UK).

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