Mod Rheumatol (2001) 11:261–273

ABSTRACTS

The 10th International Rheumatology Symposium in Tokyo "2001 Frontier of Rheumatology"

March 23–24, 2001, Tokyo, Japan

Kazuhiko Yamamoto, Organizing Committee Chairman

*Sponsored by Japan Rheumatism Association / Japan Rheumatism Foundation

Genetic approach to human SLE

Timothy W. Behrens

Center for Immunology, University of Minnesota, Minneapolis, MN, USA

Systemic lupus erythematosus (SLE) is a chronic, debilitating autoimmune disease caused by a complex interaction of genetic and environmental factors. Efforts in our laboratory and several others are aimed at identifying the genes that predispose individuals to SLE. While genome screens have identified several interesting regions, our fine-mapping efforts are currently focused on two regions – chromosome 1q41–42 and the HLA locus on 6p21.

Three SLE genome screens and a targeted marker study found evidence for a genetic linkage at D1s229 in the 1q41–42 region. This region, which lies just centromeric to the poly ADP ribose polymerase (*PARP*) gene, is syntenic to the SLE1d locus recently identified in the NZM2410 murine model of SLE by W. Wakeland and colleagues. In order to characterize this region further in our family collection, 12 microsatellite markers flanking D1s229 were genotyped in 211 SLE sib-pair families and 123 SLE trio families. The best evidence for linkage was found at D1s2616 (LOD = 1.15) using multipoint nonparametric analyses with GENEHUNTER PLUS. A two-marker multiallele transmission distortion test (TDT) revealed evidence for significant linkage disequilibrium in this region, with a three-marker haplotype (D1s229–D1s227–D1s2616) demonstrating significant transmission distortion in the family collection ($P < 0.000096$). The presence of genetic linkage, the potential synteny of this region with a murine SLE locus, and the evidence for LD warrant further gene discovery efforts in the 1q41–42 interval.

Association studies have implicated HLA (6p21) as an important genetic locus in SLE. Supporting these findings, three independent genome screens have found suggestive evidence for genetic linkage in the 6p21 region. Recent fine mapping of the 6p21 region using 211 SLE sib-pair families revealed strong evidence for genetic linkage (LOD = 3.30) within the HLA. However, owing to the high density of genes and extensive linkage disequilibrium across the HLA it has proven difficult to identify relevant susceptibility genes.

Therefore, the HLA is being genetically dissected using a dense map of over 50 known, and several newly developed, polymorphic microsatellite markers distributed across the length of the region. Analysis thus far of 45 markers genotyped in 334 SLE families has identified two extended haplotypes (termed HR1 and HR2) showing strong evidence of linkage and transmission distortion.

Families with founder individuals bearing HR1 and/or HR2 account for the linkage seen at the HLA within our population (LOD = 3.45 for families with HR1 and/or HR2, LOD = 1.5 for families without HR1 or HR2). Both HR1 and HR2 exhibit transmission distortion across the HLA, but the most significant transmission distortion was observed at the Class II/III boundary (HR1 TDT *P* value = 0.005, HR2 TDT *P* value = 0.001) and at the Class III/I boundary (HR1 TDT *P* value = 0.005, HR2 *P* value = 0.007). HR1 and/or HR2 is present in over half of the families in the study. It is likely that HR1 and HR2 harbor SLE susceptibility gene(s) with a major effect. Additional marker analyses within these families should allow narrowing of the susceptibility intervals using a combination of TDT and ancestral recombinant haplotype analyses.

List of representative publications

1. Gaffney PM, Ortmann WA, Selby SA, Shark KB, Ockenden TC, Rohlf KE, et al. Genome screening in human systemic lupus erythematosus: results from a second Minnesota cohort and combined analyses of 187 sib-pair families. Am J Hum Genet 2000;66:547–56.

*The International Rheumatology Symposium was organized by the Japan Rheumatism Foundation until 2000. The 2001 symposium was jointly organized by the Japan Rheumatism Association and the Japan Rheumatism Foundation

2. Gaffney PM, Kearns GM, Shark KB, Ortmann WA, Selby SA, Malmgren ML, et al. A genome-wide search for susceptibility genes in human systemic lupus ery-thematosus sib-pair families. Proc Natl Acad Sci USA 1998;95:14875–9.
3. Harley JB, Moser KL, Gaffney PM, Behrens TW. The genetics of human systemic lupus erythematosus. Curr Opin Immunol 1998;10:690–6.

Genetic study of SLE: lessons from mouse models

Sachiko Hirose

Department of Pathology, Juntendo University School of Medicine, Tokyo, Japan

Systemic lupus erythematosus (SLE) is a multigenic disease associated with IgG hypergammaglobulinemia, IgG antinuclear antibodies, and immune complex-type glomerulonephritis. Major genes that predispose to SLE are related to key events in the pathogenesis, and may involve a variety of genes in the immune system. Recently, the application of the polymerase chain reaction and the availability of maps of microsatellites have facilitated a genome-wide scan to define the number and locations of genes for complex traits. However, the extreme diversity of the human genome and the complexity of multifactorial inheritance have delayed completion of a genome-wide analysis of susceptibility loci for human SLE. In this respect, studies of polymorphisms and the functions of candidate genes, suggested based on studies of murine models, may be followed by studies on SLE patients. Here, I focus on the regulatory region polymorphisms of candidate genes, and discuss their possible involvement in the development of SLE.

The first report concerning the association of regulatory region polymorphism and SLE was for the *TNF-a* gene. As in humans, the murine *TNF-a* gene is located within the MHC region. A unique polymorphism in a simple tandem repeat sequence is identified in the promoter region of the *TNF-a* gene, and the NZW strain carries a unique *TNF-a* allele, capable of producing only limited amounts of *TNF-a*. Because administration of *TNF-a* induces a significant delay in the onset of nephritis in (NZB × NZW) F1 mice, the NZW *TNF-a* gene may be involved in the pathogenesis of SLE in these mice. However, the mechanism for the association of downregulating *TNF-a* levels and SLE is unknown.

We recently found that there were promoter-region polymorphisms of the *FcgRIIB* gene with several nucleotide deletions. These deletions were detected not only in NZB, but also in other SLE-prone BXSB and MRL, and autoimmune diabetes-prone NOD strains. Furthermore, these polymorphisms showed a significant association with IgG immune responses, including autoantibodies, in association with marked downregulation of *FcgRIIB1* expression on germinal center B cells. The B1 isoform of *FcgRIIB* is a key inhibitor of B cell antigen receptor signaling, suggesting that functional defects due to the regulatory region polymorphisms of the *FcgRIIB* gene result in enhanced B cell responses through germinal center B cells downregulating *FcgRIIB1* upon antigen stimulation. Thus, these polymorphisms seem to function as a kind of immune regulatory gene, and may have been evolutionarily selected and maintained for effective natural defense against pathogens, which in turn eventually form one basis of autoimmune susceptibility. Based on the above findings, together with our recent data, we suggest that there are several kinds of genetic polymorphisms not only of structural but also of regulatory regions for functioning molecules which are important for immune homeostasis, and that the incidental combination of these polymorphisms may form the basis of SLE-susceptibility. A thorough understanding of the genetic basis for SLE will provide clues about the pathogenesis, and then better prophylactic and therapeutic clinical approaches can be designed.

References

1. Fujimura T, et al. Dissection of the effects of tumor necrosis factor-a and class II gene polymorphisms within the MHC on murine systemic lupus erythematosus. Int Immunol 1998;10:1467–72.
2. Jiang Y, et al. Genetically determined aberrant down-regulation of *FcgRIIB1* in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. Int Immunol 1999;11:1685–91.
3. Jiang Y, et al. Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. Immunogenetics 2000;51:429–35.
4. Hirose S, et al. Genetic aspects of inherent B-cell abnormalities associated with SLE and B-cell malignancy: lessons from New Zealand mouse models. Int Rev Immunol 2000;19:389–421.

Gene therapy: recent progress in gene transfer technology

Takashi Shimada

Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan

Gene therapy is defined as the treatment of disease by the transfer of genes into patients. This strategy was originally developed to establish a definitive therapy for genetic diseases. Recently, acquired diseases such as cancer, vascular disorders, and rheumatoid arthritis have also been considered to be important targets for gene therapy. To date, although more than 300 clinical protocols have been carried out involving a total of more than 3000 patients, the clinical efficacy of gene therapy has been demonstrated in only a small number of these.

The establishment of a safe and efficient gene delivery system is the most important element in the success of gene therapy. Moloney murine leukemic virus (MLV)-based retroviral vectors and adenovirus vectors have been widely used in patients. Retrovirus stably integrates the genetic information into chromosomal DNA. However, MLV can infect only actively dividing cells, and thus the utility of MLV vectors for in vivo protocols is very limited. Adenovirus is capable of infecting many different types of cell, including nondividing cells, with an extremely high efficiency.

However, adenovirus-mediated gene expression is transient, and the current adenoviral vectors induce both humoral and cell-mediated immunoreactions. Accordingly, neuronal cells and hematopoietic stem cells, both of which are important targets in many gene therapy protocols, are not treated efficiently by the current vector systems.

New viral vectors derived from adeno-associated virus (AAV) and human immunodeficiency virus (HIV) have attracted considerable attention in the attempt to overcome these problems. AAV is nonpathogenic and is capable of being integrated into the defined chromosomal locus. AAV vectors are shown to be extremely useful for gene transfer into neuronal and muscle cells. HIV-based vectors bind specifically to the CD4 antigen, and are capable of targeted gene transfer into CD4+ cells. The strict cell specificity of HIV vectors will likely prove important in the development of gene therapy for AIDS. Recent research has demonstrated that HIV vectors pseudotyped with a vesicular stomatitis G envelope can stably transduce various nondividing cells. Therefore, if the safety concerns can be overcome, HIV vectors should be useful for gene therapy for both genetic and acquired diseases. A major drawback of these two vector systems is the lack of an efficient production system. The establishment of strategies for large-scale preparation of high-titer vector particles is thus urgently required.

Targeted gene transfer into specific cell types is a key technological requirement for the next generation of gene therapy. Transcriptional targeting using tissue-specific promoter/enhancer elements is one possible approach. For a more general approach, a viral envelope of capsid proteins could be engineered to bind specifically to target cells. Alternatively, the target cells could be modified by introducing the receptor gene that encodes for the vector particles, a process that would make them susceptible to vector infection.

The repair of genetic defects is the ultimate goal of gene therapy. The efficiency of homologous recombination currently falls far below the therapeutic level. One possibility involving forced mismatch repair using chimeric oligonucleotides has recently been examined. As a practical gene targeting approach, AAV-mediated gene insertion into a defined locus has also been attempted.

The future of gene therapy is promising, but we are still at the very initial stages. Further development of gene transfer vectors will be required for the successful application of this innovative technology to treat a wide variety of diseases.

A new paradigm for treatment of rheumatoid arthritis

Peter E. Lipsky

National Institute of Arthritis and Musculoskeletal and Skin Disease, NIH, Bethesda, MD, USA

Traditionally, therapy for rheumatoid arthritis has been empiric, since little was known about the etiology of the disease or the pathogenic mechanisms involved in chronic inflammation and tissue damage. Moreover, the available therapies were nonspecific, and were used with the intent of suppressing inflammation and/or immune responses. As a result, the therapy was often unsuccessful, with incomplete control of the signs and symptoms of inflammation and minimal impact on progressive disability.

In the past two decades, intensive investigation has provided new insights into the pathogenesis of rheumatoid arthritis, although the etiology remains unknown. Many of the steps involved in the chronic inflammation of rheumatoid arthritis have been delineated, revealing a complex pattern involving a variety of cell types producing a large number of proinflammatory molecules. One proinflammatory cytokine that is produced in abundance in rheumatoid synovitis is tumor necrosis factor (TNF). This cytokine contributes to many of the components of rheumatoid inflammation, including the upregulation of adhesion molecules by endothelial cells, the induction of proinflammatory cytokines and clemokines by a variety of cell types, the induction of enzymes capable of generating mediators that contribute to inflammation, such as cyclooxygenase-2, and the costimulation of hepatocytes to produce acute-phase reactants. Experiments employing animal models of arthritis have confirmed a role for this cytokine in inflammatory synovitis. Two agents have been developed to neutralized TNF in patients. The first is a TNF receptor IqG1 contruct that binds both TNF and lymphotoxin. The second is a chimeric (mouse Fv and human IqG1 Fc) monoclonal antibody that is specific for TNF. Both molecules have been shown to be effective in treating rheumatoid arthritis, suppressing signs and symptoms, normalizing acute-phase reactants, and retarding or inhibiting radiographic progression. Notably, there seems to be a dichotomy between the capacity of these agents to controls signs and symptoms and radiographic progression, in that progressive damage to cartilage and bone is inhibited even in patients who do not respond clinically. Adverse events appear to be modest, although concerns about an increased susceptibility to infection or malignancy remain. Although these are potent therapies, it is clear that they are suppressive and not curative, as disease activity returns once therapy is discontinued, even after prolonged treatment.

A number of issues about the use of TNF neutralizing agents remain unclear, including whether concomitant therapy is necessary, and if so what is the best cotherapy. In addition, the long-term safety and efficacy remain unknown, as do the characteristics of patients who respond and those who are resistant. Despite these caveats, TNF neutralizing therapy is highly effective in patients with rheumatoid arthritis. It represents the first rational targeted therapy for this disease.

References

1. Lipsky PE, Van Der Heijde DMFM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000;343:1594–602.

2. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000;343: 1586–93.

Role of HLA-DR in autoimmune arthritis

Andrew H. Kang · Linda K. Myers · David Brand · John M. Stuart · Edward F. Rosloniec

Departments of Medicine and Pediatrics, University of Tennessee College of Medicine and Research Service, Veterans Affairs Medical Center, Memphis, TN, USA

Although the etiology of rheumatoid arthritis (RA) remains uncertain, the preponderance of evidence suggests that it is mediated by an antigen-driven autoimmune process. This notion is supported by the fact that susceptibility to RA is strongly associated with the expression of specific human leucocyte antigen (HLA) class II alleles, especially *HLA-DR1* and *DR4*. However, it is not clear what the role of these class II molecules is in the disease process, or which target antigen(s) is the focus of the autoimmune response. Since the pathology of RA is predominantly a chronic inflammation of diarthroidial joints, it has been proposed that the autoimmunity in RA is specific for the protein(s) found in these joints. The fact that joint inflammation subsides upon surgical removal of articular cartilage, or in end-stage disease where articular cartilage has been completely destroyed, is consistent with this view. Type II collagen (CII) has received considerable attention as a candidate autoantigen since it is the predominant protein of articular cartilage, and autoimmunity to CII is commonly detected in patients with RA. Furthermore, immunization with CII induces an autoimmune arthritis resembling RA in several species of animals, which is known as collagen-induced arthritis (CIA). Although it has not been proven that autoimmunity to CII initiates RA, it seems clear that at least some of the autoimmunity in RA is directed towards CII. The problem, however, has been in determining the relationship between immunity to CII and the role of the class II alleles that confer susceptibility to RA. The recent development of transgenic (Tg) mice expressing HLA class II molecules has made it possible to address this question experimentally. Our recent studies with HLA-DR Tg mice show that DR1 (DRB1*0101) and DR4 (DRB1*0401) can bind and present peptides derived from human CII, and that these transgenic DR molecules confer susceptibility to experimental autoimmune arthritis. Using proliferation and cytokine assays, we have identified CII 262–270 (GFKGEQGPK) as the immunodominant T cell determinant core for presentation by HLA-DR1 and DR4. Based on these data, we have developed synthetic analog peptides containing substitutions at selected residues that possess altered peptide ligand (APL) properties and can downregulate the immune response to CII and CIA in DR Tg mice.

On this basis, we conclude that the autoimmune response to CII, clearly present in many cases of RA, is a significant contributory factor in the pathogenesis of the disease, and that if the CII-specific immunity can be disrupted, the inflammation can be ameliorated.

A perspective on the treatment of rheumatoid arthritis in 2001

Lee S. Simon

Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

The etiology and pathogenesis of diseases such as rheumatoid arthritis are just beginning to be elucidated. Unfortunately, at this juncture there has been no convincing evidence that any presently available remittive therapy for rheumatoid arthritis will substantially alter the ultimate outcome. New therapies, including biological response modifiers such as the inhibitors of *TNF*-α (infliximab, entanercept), an inhibitor of IL1-ra (anakinra), and immunomodulators such as leflunomide, along with other therapies with a longer history of use, including methotrexate and sulfasalazine, have all been shown to improve the signs and symptoms of inflammation about equally, as well as being approximately equal in improving the health-related quality of life (HRQOL). In addition, all these therapies have been shown to decrease X-ray progression of damage similarly in the short term (up to 2 years). With these data, the treatment of RA has generally become more aggressive, with the earlier use of substantial disease-modifying drugs. In addition, many of the drugs previously widely used have been abandoned, e.g., injectable gold salts, penicillamine, azathioprine, and cyclophosphomide, owing to risk–benefit issues. Of course, even these newer therapies have specific advantages and disadvantages.

The potential advantages of the "biological agents" (biological response modifiers) include their relatively rapid onset of effect when intervention is successful. This is quite an advance over the slower delayed responses observed with therapies such as leflunomide (which may take up to 4 weeks to demonstrate a substantial anti-inflammatory effect), or methotrexate or sulfasalazine, which might take up to 8 weeks for "acute benefit." The exquisite specificity of many of the biological response modifiers has allowed a further "teasing-out" of the inflammatory cascade, giving a further insight into the pathogenesis of the disease state. Their use has also taught us about the redundancy of the cytokine network. There are clearly some patients who begin to lose their effective response to some of these therapies. Sometimes this is due to the natural ability of the body to develop an immune response to the therapeutic intervention, thus neutralizing its effects. Alternatively, but equally as powerful as neutralization, is the "bypassing" of the effect through another pathway in the inflammatory cytokine pathway which may further "drive" the systemic inflammatory disease.

The benefits of drugs such as leflunomide and methotrexate are their lower cost compared with the biological

modifiers, oral therapy (at least with methotrexate at <20 mg per week), and somewhat greater ease of use. Leflunomide, methotrexate, and the biological response modifiers such as entenercept and infliximab have all shown 2-year data with a similar durable response in terms of the signs and symptoms of the disease, HRQOL, and X-ray progression.

There are specific disadvantages with the biological agents. The therapies require some form of parenteral administration. In the age of managed care, this is not a popular way to introduce a new cost-effective therapeutic intervention for a chronic illness. Thus, the costs of therapy, not just the hidden development costs, which of course are substantial, are quite difficult to justify given the lack of an ideal response (cure) with therapy. Therapy with TNF-α inhibitors has been associated with a decrease in patient response to infections, including such intracellular infections as tuberculosis, listeriosis, and some fungal diseases. Therapy with IL-1ra was associated with significant infections in 4% of those treated. However, not all delayed problems are seen with biological interventions only. There have now been 15 cases of non-Hodgkin's lymphoma and Hodgkin's lymphoma reported in patients with RA treated chronically with methotrexate. Leflunomide and the TNF-α inhibitors have not been available long enough for their potential for malignancy with chronic long-term use to be known. Combination therapy with leflunomide and methotrexate induces potential liver toxicity more frequently than leflunomide alone.

As in cancer therapy, the "biological" agents may serve as potential induction therapies to gain initial control of an inflammatory disease, with the subsequent utilization of other therapies, sequentially or in combination, which might be less costly once the disease has quieted. The biological response modifiers could be used to reinduce a "remission" to treat flares of the disease. Given the very specific effects of some of the biological agents, combinations of these therapies may be very powerful tools. Finally, these therapies have helped tremendously by serving as modulating probes which have allowed us a greater understanding of the critical pathways within the various inflammatory and cytokine pathways. Before these agents were available, we knew very little of the central role of TNFα within the cytokine pathways driving inflammation in rheumatoid arthritis.

All these new agents may be at their best when used early in the course of the illness. Their use at the initiation of disease is probably very different from their effect 15 years later. Certainly, the new COX-2-specific imhibitors improve the risk–benefit ratio of the drugs which have served to palliate pain and inflammation; however, these drugs have never been shown to have a substantial effect on disease progression. Almost all of the described trials with the newer disease-modifying drugs, which include almost all the available data, have been designed to be of short duration, and were basically done to achieve the approval of regulatory bodies. The longest studies have lasted 2 years. We have learned that the best way to evaluate the effects of drugs in chronic disease include trials of longer duration. Only in this manner can efficacy be determined. Short-term trials with drugs may give some understanding of their toxicity, but we are cannot be sure that short-term trials with these agents will give the same information about toxic effects as longer trials.

Thus, at this point it is important to look for future directions. Clearly, investigators have to improve their trial designs. Prospective, randomized, controlled clinical trials are of critical importance, along with well-designed and detail-oriented dosing and dose-scheduling preliminary work to ensure that the subsequent randomized trials are created appropriately. It is critical to design these trials around a definable patient population with an active and potentially responsive disease. Outcome measures should be standardized across trials. An intention-to-treat analysis should be done as well as a "completers" analysis. With what is known about delayed toxic reactions, long-term follow-up (>5 years), perhaps as open-label trials, is also essential. Finally, given the precision of the effects of these drugs, it is possible that combination therapy with several different agents will prove to be better than any one alone.

References

General

Mikuls T, Moreland L. The treatment of rheumatoid arthritis: a review of recent clinical trials. Curr Rheumatol Rep 1999;1:135–8.

Furst DE, Breedveld FC, Burmester GR, Crofford JJ, Emery P, Feldmann M, et al. Updated consensus statement on tumour necrosis factor blocking agents for the treatment of rheumatoid arthritis (May 2000). Ann Rheum Dis 2000;59 Suppl 1:1–2.

Strand V. Recent advances in the treatment of rheumatoid arthritis. Clin Cornerstone 1999;2(2):38–50.

Simon LS, Yocum D. New and future drug therapies for rheumatoid arthritis. Rheumatology (Oxford) 2000;39 Suppl 1:36–42.

Leung H, Hurley F, Strand V. Issues involved in a metaanalysis of rheumatoid arthritis radiographic progression. Analysis issues. J Rheumatol 2000;27:544–8.

Leflunomide

Strand V, Cohen S, Schiff M, Weaver A, Fleischmann R, Cannon G, et al. Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Group. Arch Intern Med 1999;159:2542–50.

Smolen JS, Kalden JR, Scott DL, Rozman B, Kvien TK, Larsen A, et al. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. European Leflunomide Study Group. Lancet 1999;353(9149):259–66.

Smolen JS, Emery P. Efficacy and safety of leflunomide in active rheumatoid arthritis. Rheumatology (Oxford) 2000;39 Suppl 1:48–56.

Prakash A, Jarvis B. Leflunomide: a review of its use in active rheumatoid arthritis. Drugs 1999;58:1137–64.

Tugwell P, Wells G, Strand V, Maetzel A, Bombardier C, Crawford B, et al. Clinical improvement as reflected in measures of function and health-related quality of life following treatment with leflunomide compared with methotrexate in patients with rheumatoid arthritis: sensitivity and relative efficiency to detect a treatment effect in a 12-month, placebo-controlled trial. Leflunomide Rheumatoid Arthritis Investigators Group. Arthritis Rheum 2000;43:506–14.

Sharp JT, Strand V, Leung H, Hurley F, Loew-Friedrich I. Treatment with leflunomide slows radiographic progression of rheumatoid arthritis: results from three randomized controlled trials of leflunomide

in patients with active rheumatoid arthritis. Leflunomide Rheumatoid Arthritis Investigators Group. Arthritis Rheum 2000;43:495–505.
Fox RI, Herrmann ML, Frangou CG, Wahl GM, Morris RE, Strand V, et al. Mechanism of action for leflunomide in rheumatoid arthritis. Clin Immunol 1999;93:198–208.

Anti-TNF-α therapy

Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. Ann Intern Med 1999;130:478–86.
Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999;340:253–9.
Moreland LW, McCabe DP, Caldwell JR, Sack M, Weisman M, Henry G, et al. Phase I/II trial of recombinant methionyl human tumor necrosis factor binding protein PEGylated dimer in patients with active refractory rheumatoid arthritis. J Rheumatol 2000;27:601–9.
Mathias SD, Colwell HH, Miller DP, Moreland LW, Buatti M, Wanke L. Health-related quality of life and functional status of patients with rheumatoid arthritis randomly assigned to receive etanercept or placebo. Clin Ther 2000;22:128–39.
Lovell DJ, Giannini EH, Reiff A, Cawkwell GD, Silverman ED, Nocton JJ, et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. N Engl J Med 2000;342:763–9.
Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997;337:141–7.
Murray KM, Dahl SL. Recombinant human tumor necrosis factor receptor (p75) Fc fusion protein (TNFR:Fc) in rheumatoid arthritis. Ann Pharmacother 1997;31:1335–8.
Cook DA, Dimick JB, Gallagher DC. Etanercept in rheumatoid arthritis. N Engl J Med 1999;340:2000.
Moreland LW. Inhibitors of tumor necrosis factor for rheumatoid arthritis. J Rheumatol 1999;26 Suppl 57:7–15.
Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000;343:1594–602.
Kavanaugh A, St Clair EW, McCune WJ, Braakman T, Lipsky P. Chimeric anti-tumor necrosis factor-α monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. J Rheumatol 2000;27:841–50.
Charles PJ, Smeenk RJ, De Jong J, Feldmann M, Maini RN. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. Arthritis Rheum 2000;43:2383–90.
Pisetsky DS. Tumor necrosis factor alpha blockers and the induction of anti-DNA autoantibodies. Arthritis Rheum 2000;43:2381–2.

IL1-ra

Schiff MH. Role of interleukin-1 and interleukin-1 receptor antagonist in the mediation of rheumatoid arthritis. Ann Rheum Dis 2000;59 Suppl 1:i103–8.
Arend WP, Guthridge CJ. Biological role of interleukin 1 receptor antagonist isoforms. Ann Rheum Dis 2000;59 Suppl 1:i60–4.
Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum 1998;41:2196–204.
Jiang Y, Genant HK, Watt I, Cobby M, Bresnihan B, Aitchison R, et al. A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. Arthritis Rheum 2000;43:1001–9.

Methotrexate

Weinblatt ME, Maier AL, Fraser PA, Coblyn JS. Longterm prospective study of methotrexate in rheumatoid arthritis: conclusion after 132 months of therapy. J Rheumatol 1998;25:238–42.
Weinblatt ME. Methotrexate in rheumatoid arthritis: toxicity issues. Br J Rheumatol 1996;35:403–5.
Kremer JM, Alarcon GS, Weinblatt ME, Kaymakcian MV, Macaluso M, Cannon GW, et al. Clinical, laboratory, radiographic, and histopathologic features of methotrexate-associated lung injury in patients with rheumatoid arthritis: a multicenter study with literature review. Arthritis Rheum 1997;40:1829–37.
Alarcon GS, Kremer JM, Macaluso M, Weinblatt ME, Cannon GW, Palmer WR, et al. Risk factors for methotrexate-induced lung injury in patients with rheumatoid arthritis. A multicenter, case-control study. Methotrexate–Lung Study Group. Ann Intern Med 1997;127:356–64.
Kremer JM, Furst DE, Weinblatt ME, Blotner SD. Significant changes in serum AST across hepatic histological biopsy grades: prospective analysis of three cohorts receiving methotrexate therapy for rheumatoid arthritis. J Rheumatol 1996;23:459–61.
Weinblatt ME, Kremer JM, Coblyn JS, Maier AL, Helfgott SM, Morrell M, et al. Pharmacokinetics, safety, and efficacy of combination treatment with methotrexate and leflunomide in patients with active rheumatoid arthritis. Arthritis Rheum 1999;42:1322–8.
Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000;343:1586–93.

Glucocorticoids

Strand V. Steroid withdrawal favours joint erosion in rheumatoid arthritis. Clin Exp Rheumatol 1999;17:519–20.

Sulafasalazine

Weinblatt ME, Reda D, Henderson W, Giobbie-Hurder A, Williams D, Diani A, et al. Sulfasalazine treatment for rheumatoid arthritis: a metaanalysis of 15 randomized trials. J Rheumatol 1999;26:2123–30.

Cyclosporin

Cush JJ, Tugwell P, Weinblatt M, Yocum D. US consensus guidelines for the use of cyclosporin A in rheumatoid arthritis. J Rheumatol 1999;26:1176–86.

Combo Rx

Tugwell P, Pincus T, Yocum D, et al. Combination therapy with cyclosporin and methotrexate in severe RA. NEJM 1995;333:137–41.
Mroczkowski PJ, Weinblatt ME, Kremer JM. Methotrexate and leflunomide combination therapy for patients with active rheumatoid arthritis. Clin Exp Rheumatol 1999;17(6 Suppl 18):S66–8.
Weinblatt ME, Dixon JA, Falchuk KR. Serious liver disease in a patient receiving methotrexate and leflunomide. Arthritis Rheum 2000;43:2609–11.
Isaacs JD, Morgan AW, Strand V. Combination biologic therapy. Clin Exp Rheumatol 1999;17(6 Suppl 18):S121–4.
O'Dell JR. Combination DMARD therapy with hydroxychloroquine, sulfasalazine, and methotrexate. Clin Exp Rheumatol 1999;17(6 Suppl 18):S53–8.
Pincus T, O'Dell JR, Kremer JM. Combination therapy with multiple disease-modifying antirheumatic drugs in rheumatoid arthritis: a preventive strategy. Ann Intern Med 1999;131:768–74.
O'Dell JR. Triple therapy with methotrexate, sulfasalazine, and hydroxychloroquine in patients with rheumatoid arthritis. Rheum Dis Clin North Am 1998;24:465–77.
O'Dell JR. Combination DMARD therapy for rheumatoid arthritis: a step closer to the goal. Ann Rheum Dis 1996;55:781–3.
O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ, et al. Treatment of rheumatoid arthritis with methotrexate alone,

sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med 1996;334:1287–91.
Kremer JM. Methotrexate and leflunomide: biochemical basis for combination therapy in the treatment of rheumatoid arthritis. Semin Arthritis Rheum 1999;29:14–26.

MTX

Shiroky JF, Frost A, Skelton JD, et al. Complications of immunosuppression associated with weekly low-dose methotrexate. J Rheumatol 1991;18:1172–5.
Ellman MH, Hurwitz H, Thomas C, et al. Lymphoma developing in a patient with RA taking low-dose weekly methotrexate. J Rheumatol 1991;18:1741–3.
Kamel OW, van de Rijn M, Weiss LM, et al. Brief report: reversible lymphomas associated with EBV occurring during methotrexate therapy for RA and dermatomyositis. NEJM 1993;328:1317–21.
Zimmer-Galler I, Lie JT. Choroidal infiltrates as the initial manifestation of lymphoma in RA after treatment with low-dose methotrexate. Mayo Clin Proc 1994;69:258–61.
Davies JMS, Kremer JM, Furst DE, et al. Lymphomatous changes during methotrexate therapy. Arthritis Rheum 1995;38:S2004.

Mechansims of autoimmunity in SLE

David S. Pisetsky

Duke University Medical Center, Durham, NC, USA

Autoimmune diseases are a diverse group of conditions characterized by immunological reactivity against normal host constituents in association with tissue inflammation and destruction. Of these diseases, systemic lupus erythematosus (SLE) has been intensively studied as a prototype of generalized or systemic autoimmunity. Among the striking features of SLE is the production of antibodies to DNA (anti-DNA). These antibodies serve as markers of diagnosis and prognosis. In addition, anti-DNA can directly mediate glomerulonephritis in lupus. The close association of anti-DNA with SLE has suggested that elucidating this response would provide important information about underlying pathogenetic mechanisms of autoimmunity.

As shown by studies on both human and murine SLE, the anti-DNA response has features of antigen drive. These features include the IgG isotype, high-affinity binding, and patterns of somatic mutation consistent with selection by DNA antigen. A key question in the etiology of this response concerns the source of this DNA. Since DNA in vivo is likely released from cells, nucleosomes have been considered to be the relevant source of this antigen, serving as large macromolecular complexes driving responses. Support for the role of nucleosomes in eliciting anti-DNA comes from the demonstration of T cell reactivity to nucleosomes, and the coexistence and temporal relationship of anti-DNA with antibodies to other chromatin components.

Despite evidence that nucleosomal DNA drives anti-DNA responses, it has been difficult to induce anti-DNA production by immunization with mammalian DNA or nucleosomes. This difficulty has spurred interest in other sources of DNA that may have greater immunogenicity. As has now been shown by in vivo and in vitro experiments, bacterial DNA, in contrast to mammalian DNA, has potent immunological properties that include cytokine induction and polyclonal B cell activation. These properties result from short-sequence motifs, called CpG motifs, or immunostimulatory sequences, that occur much more commonly in bacterial than in mammalian DNA. Because of its content of CpG motifs, bacterial DNA has adjuvant properties and can induce significant anti-DNA responses in mice under conditions in which mammalian DNA is inactive.

Interestingly, recent studies have suggested that mammalian DNA itself is immunologically active, although its activities lead to inhibition of immune responses. Thus, in vitro studies have shown that mammalian DNA can inhibit the immunostimulatory activities of bacterial DNA. This inhibition is also observed with synthetic oligonucleotides, with these compounds being able to suppress the activity of LPS as well as bacterial DNA in certain systems. While the sequences in mammalian DNA causing inhibition have not yet been determined, these observations raise the possibility that self-DNA can play a countervailing role and limit immune responses in settings of inflammation or infection.

These considerations suggest that DNA can exert powerful effects on the immune system, with the species origin and sequence determining whether they are stimulatory or inhibitory. Studies on the etiology of SLE must therefore address whether anti-DNA responses result from the action of an immunostimulatory foreign antigen (bacterial DNA) or the action of an inert or inhibitory self-antigen (mammalian DNA). In either case, it is likely that genetically determined factors of the host strongly influence the nature of these responses. Studies are in progress to identify these factors and their interplay with foreign and self-DNA.

Reference

1. Pisetsky DS, Reich CF. Inhibition of murine macrophage IL-12 production by natural and synthetic DNA. Clin Immunol 2000;96: 198–204.

Autoimmune diseases in PD-1 deficiency: a negative regulation in peripheral tolerance

Tasuku Honjo

Department of Medical Chemistry, Kyoto University, Faculty of Medicine, Kyoto, Japan

PD-1, a 55 kD transmembrane protein containing an immunoreceptor tyrosine-based inhibitory motif, is induced in lymphocytes and monocytic cells following activation. Aged C57BL/6(B6)-PD-1−/− congenic mice spontaneously developed characteristic lupus-like proliferative arthritis and glomerulonephritis with predominant IgG3 deposition, which were markedly accelerated by the introduction of a Fas mutation (lpr). The introduction of a PD-1 null mutation into 2C-TCR (anti-H-2Ld) transgenic mice

with an H-2b/d background resulted in a chronic and systemic graft-versus-host-like disease. Furthermore, CD8+2C-TCR+PD-1−/− T cell exhibited markedly augmented proliferation in vitro in response to H-2d allogenic cells.

Collectively, it is suggested that PD-1 is involved in the maintenance of peripheral self-tolerance by serving as a negative regulator of immune responses.

Current trends in lupus nephritis

Dimitrios T. Boumpas

University of Crete, Greece

Glomerulonephritis is a major cause of morbidity in systemic lupus erythematosus (SLE). In this disease, immune complex formation/deposition in the kidney results in intraglomerular inflammation with the recruitment of leukocytes, and the activation and proliferation of resident renal cells. Intense injury may destroy resident renal cells by necrosis or apoptosis, resulting in fibrinoid necrosis. When the injury is less intense, endocapillary cells respond by proliferating and the production of extracellular matrix (proliferative lesions).

Determination of disease severity

Renal biopsy, examination of the urine sediment, and measurement of C3 levels (and to a lesser degree of anti-DNA titers) are essential for the management of lupus nephritis. Treatment depends on the severity of the disease, and disease severity depends on the presense or absence of high-risk factors. These include demographic (male gender, black race), clinical (failure to achieve response or marked delay in response, multiple relapses, pregnancy), laboratory (impaired renal function, severe anemia with hematocrit less than 26%), and histological (mixed membranous and proliferative or proliferative nephritis, cellular crescents and/or fibrinoid necrosis, moderate to high degrees of interstitial fibrosis and/or tubular atrophy) features.

Treatment

Patients with mild proliferative disease without risk factors are usually treated with corticosteroids alone or in combination with azathioprine. If the disease does not remit within 3–4 months, cytotoxic drugs such as cyclophosphamide, or newer agents such as mycophenolate, may be tried. For patients with moderate to severe proliferative nephritis, controlled trials have shown that pulse cyclophosphamide is the treatment of choice. Long-term follow-up of patients participating in these controlled trials suggests that combining pulse cyclophosphamide with pulse methylprednisolone increases efficacy but not toxicity. For lupus membranous nephritis, steroids, pulse cyclophosphamide therapy, or cyclosporine may be used. Relapse rates are high when cyclosporine is discontinued. In addition to immunosuppressive therapy, aggressive management of co-morbid conditions (hypertension, dyslipidemia, osteoporosis) is of paramount importance.

Response rates and flares

Rates of clinical response and flares vary in different studies according to the type of nephritis, treatment regimen, duration of therapy, and definition of the clinical response and flare used. Flares pose a significant problem because of the risk of renal function deterioration due to cumulative damage, as well as cumulative toxicities due to additional immunosuppressive therapy. Patients with nephritic flares (defined as an increase in plasma creatinine level and the reappearance of active nephritic urinary sediment) are more likely to progress to end-stage renal disease in spite of additional immunosuppressive therapy.

Side effects

Pulse cyclophosphamide is associated with an increased risk of herpes zoster infections in the short term, and with sustained amenorrhea or azoospermia on the long term. Gonadotropin-releasing hormone agonist (GnRH-a) may prevent accelerated recruitment and the depletion of ovarian follicles (via suppression of the gonadotrophin production in the pituitary gland) and therefore protect against premature ovarian failure. In a small case series, testosterone was found to be effective in preserving fertility in patients with nephrotic syndrome treated with a short course of cyclophosphamide.

New approaches

A recent controlled study reported that mycophenolate mofetil is equally as effective as a regimen of oral cyclophosphamide and azathioprine used sequentially in patients with proliferative lupus nephritis. However, in this study the follow-up was short, the patients had relatively mild diseases, and patients with high-risk factors were not included. A contolled study comparing mycophenolate mofetil with pulse cyclophosphamide is in progress. The induction of a response with cyclophosphamide followed by maintenance with agents such as azathioprine or cyclosporine is under investigation. Other investigations are exploring the therapeutic potential of high-dose, immunoablative cyclophosphamide therapy alone or in combination with autologous stem-cell transplantation, low-dose cyclophosphamide in combination with nucleoside analogs, or biological response modifiers. High-dose cyclophosphamide or combinations of low doses of fludarabine may result in profound bone marrow and immune suppression. Combinations of cyclophosphamide with biological response modifiers have shown encouraging results in preclinical animal studies, and may provide a major breakthrough in the treatment of severe lupus, similar to the introduction of cytotoxic agents a few decades ago.

Undergraduate education in rheumatology: ILAR-UMER 2000 Project

J. Dequeker

Department of Rheumatology, University Hospitals KU Leuven, Leuven, Belgium

Nearly a quarter of all consultations in primary care are about the rheumatic disease, yet undergraduate education in rheumatology is underdeveloped all over the world and does not get the attention it deserves. This has important consequences for the early diagnosis of rheumatic diseases and the proper care of rheumatic patients in general, and for the esteem of rheumatology as a profession.

Because the high prevalence and impact of rheumatic diseases are not reflected in medical curricula, the International League of Associations for Rheumatology (ILAR) developed the Undergraduate Medical Education in Rheumatology (UMER) 2000 Project.

The project embodies three fundamental concepts: (1) to convince medical faculties and schools educating health professionals world-wide that skills in examination, and a knowledge of the management of musculoskeletal diseases and attitudes to disability are the basis of good medical practice; (2) that rheumatology is valuable for acquiring skills in problem solving, clinical reasoning, and understanding the basis genetic, immunological, and biochemical mechanisms, as illustrated by rheumatic diseases; (3) the orientation of these programs to the needs of individual patients in the context of the population at large, knowing that 20% of all primary care consultations involve musculoskeletal diseases.

Rheumatology practice conditions offer excellent vehicles for teaching attitudes, skills, and knowledge that have relevance and implications far beyond rheumatology as a specialty subject.

Many deans and other educators in medical and health professional schools are aware of changes in epidemiology due to aging and to the control of infectious diseases; they are aware of the enormous increase in knowledge as well as changes in medical practice, and feel obliged to change the curriculum. The rheumatological community has to appreciate this transition and take the present opportunity seriously. Rheumatology may help medical and health professional faculties to provide better clinical education for the emerging physician and health professional because of our special place in medicine, i.e., in areas that overlap with other disciplines dealing with acute and chronic diseases, and because of our clinical diagnostic approach and knowledge of pathophysiological mechanisms involving immunology, genetics, inflammation, metabolic disorders, and teamwork with health professionals.

Reference

1. Dequeker J, Rasker H. High prevalence and impact of rheumatic diseases is not reflected in the medical curriculum: the ILAR Undergraduate Medical Education in Rheumatology (UMER) 2000 Project. Together everybody achieves more. J Rheumatol 1998;25: 1037–40.

Attitude education in rheumatology

J.J. Rasker

Department of Rheumatology and Communication Sciences, University Twente, Enschede, The Netherlands

In medical education, clinical competence is often mentioned as the main objective. Medical competence is the complex of knowledge, skills, and attitudes necessary to be a good doctor. Much attention is given to knowledge and skills, but in most medical curricula, little attention is given to explicit training of attitudes.

Attitude is a combination of three elements: opinions, feelings, and the inclination to react positively or negatively to something or somebody. The resulting behavior is the only visible part. Thus, the attitude of a doctor is visible during contact with patients and colleagues, but also includes aspects such as scientific attitude and a willingness for lifelong study.

Without proper education, there is a high risk that the patient-oriented attitude with which students enter university will get lost, and students perceive that they become more cynical.

This process, also called dehumanizing, is attributed to the predominantly biomedical orientation of the medical curriculum, and the role models (examples given by doctors) in the clinical phase.

Longitudinal studies show that without direct training, dehumanizing will indeed occur, in both the opinions and behavior of medical students, although students with certain personality characteristics seem not to change.

A patient-oriented attitude is necessary on the one hand because it results in greater patient satisfaction, while on the other hand as it improves patient compliance to the advice of the doctor.

To some extent, attitude has already been defined before medical education starts; during medical education it is partly an autonomic process, but in part it can be trained, and thus it needs to receive attention from the very beginning of medical education.

During recent years, attitude education has changed: apart from teaching the "right attitude" (which means patient-directed working style, etc.) "awareness" is now the central concept, in particular awareness of one's own attitude (including emotions, norms, values, and (pre) judgment regarding medical behavior). Only after becoming aware does one know where one's attitude should be changed, and people can change their own attitude.

Aims should be formulated for attitude education. These will differ from culture to culture, and should include medical aspects, scientific aspects, personal aspects, and aspects related to society and health care systems.

The assessment of attitudes during education is important. This can be done through an evaluation by educators,

but it is better done by peers and by (simulated) patients, since this is a more realistic situation. To measure patient-oriented attitudes, a "doctor–patient" scale can be applied. The major drawback of using this as a test is that doctors will fill in what they believe to be socially desirable. The scale also shows clear differences between men and women: women show more empathy, but such differences become smaller during internships.

The attitude to disabled people is often that they are seen as second-class citizens, and regarded sociologically and psychologically as a child. Contact with disabled people, particularly disabled people who are not patients, will lead to more positive attitudes, as will personal experience, e.g., by asking a student to use a wheelchair for 2 days.

It has been shown that the attitude to disability can improve in medical students over their years of training. However, a lot still needs to be done to find the right methods to train attitudes and measure the effect of such an education. Ideally, such observations should also be carried out during specialization, and even during the work as a doctor, but this ideal is still far away.

Reference

1. Rasker JJ. Attitude education in rheumatology. J Rheumatol 1999;26 Suppl 55:41–3.

Medical education and postgraduate training program in rheumatology in Japan

Shogo Kano

Jichi Medical School, Tochigi, Japan

Among the 80 medical schools in Japan, only a few have an independent department of rheumatology for undergraduate medical education. In most medical schools, rheumatology is taught in lectures on medicine or orthopedic surgery. Recently, an increasing number of rheumatology specialists have been appointed as professors and chairpersons of Departments of Medicine, or of Orthopedic Surgery, as well as of Departments of Rheumatology. These professors have established well-organized courses of lectures on rheumatology and clerkship courses in clinical rheumatology.

Patients with rheumatoid arthritis or other rheumatic diseases were traditionally treated by general physicians, orthopedic surgeons, or internists who were interested in connective tissue diseases. In 1996, the Ministry of Health and Welfare, the Government of Japan, formally approved rheumatology as a subspecialty in medical practice.

The Japanese Board of Rheumatology (JBR) was established in 1987 as a standing committee of the Japan Rheumatism Association (JRA). The mission of the Board is to improve research and education in rheumatology, and to enhance the quality of health care of patients with rheumatic disorders in Japan, by maintaining high standards for certifying rheumatologists who possess the knowledge, skills, and clinical experience essential for excellent care.

To be admitted to the Certification Examination in Rheumatology, candidates must (a) have completed at least 3 years of postgraduate clinical training in rheumatology in an accredited program, (b) have been an active member of the JRA for more than 5 years, and (c) have at least 30 units of CME credits in rheumatology. The validity of certificates issued by the JBR lasts for 5 years. Recertification will be issued upon application if the candidate has gained the required 60 units of CME credits in the preceding 5 years and remained an active member of the JRA. There are 2830 certified rheumatologists in Japan as of March 2000. Certification is not a requirement to requirement to practice rheumatology in Japan.

Osteoprotegerin (OPG) and OPG-ligand as regulators of osteoclast activity

Ulrich Feige

Inflammation Group, Department of Pharmacology/Pathology, Amgen, Thousand Oaks, CA, USA

Bone hemostasis and remodelling are controlled by the coupled activity of bone-forming osteoblasts and bone-eroding osteoclasts. Osteoclast activity is regulated both at the level of osteoclast maturation from precursors and at the level of activity of mature osteoclasts by the balance between levels of osteoprotegerin ligand (OPG-L) and oteoprotegerin (OPG). OPG and OPGL are novel members of the TNF receptor and TNF superfamilies, respectively.[1,2] OPG ligand (OPG-L) is produced by osteoblasts and marrow stromal cells in response to bone-resorptive agents such as PTH, vitamin D, IL-1, or TNF-a.[2] OPG-L is highly expressed in osteoblast/stromal cells and activated T cells.[2–5] The interaction of OPG-L with its receptor (RANK[4]) promotes the differentiation of osteoclast precursors and the activation of mature osteoclasts, thus increasing osteoclast numbers and enhancing bone resorption.[2,6] Mice injected with OPG-L show systemic hypercalcaemia.[2] OPG-L is essential for the formation of osteoclasts, as indicated by their complete absence in OPGL−/− mice.[3,7] OPG blocks these processes by binding and inactivating OPG-L. Excess circulating OPG levels, as in transgenic mice or mice treated with recombinant OPG, result in markedly reduced numbers of osteoclasts and inhibit the ability of hormones and cytokines to increase bone resorption.[1,8] In contrast, OPG−/− mice develop early-onset osteporosis.[7]

Normal mice bearing colon-26 tumors develop increases in both parathyroid hormone-related protein (PTHrP) and plasma PTHrP, marked hypercalcemia, and increased bone resorption. OPG given either at the onset of hypercalcemia or after it had occurred blocked tumor-induced increases in bone resorption and hypercalcemia, and rapidly normalized blood ionized calcium.[9]

T cells are spontaneously activated in ctla-4−/− mice. These mice exhibit extended resorption of bone which

could be inhibited by OPG treatment.[5] Similarly, in diseases characterized by systemic activation of T cells, such as adjuvant arthritis in rats, OPG-L-mediated increases in osteoclastogenesis and bone loss are observed, both of which can be counteracted by treatment with OPG.[5] Interestingly, full preservation of bone has been observed, although no effect on inflammatory processes was seen, following OPG treatment in these rats. A closer analysis of the effects of OPG treatment on osteoclasts in vivo revealed that withdrawal of OPG-L results in osteoclast apoptosis within 48 h.[10] The results of treatment with OPG will be compared and contrasted with the effects of treatment with anti-IL-1, anti-TNF, or combinations which resulted in the inhibition of inflammatory as well as bone and cartilage destructive processes.[11]

References

1. Simonet et al. Cell 1997;89:309–19.
2. Lacey et al. Cell 1998;93:165–76.
3. Kong et al. Nature 1999;397:315–23.
4. Anderson et al. Nature 1997;390:175–9.
5. Kong et al. Nature 1999;402:304–9.
6. Yasuda et al. PNAS 1998;95:3597–602.
7. Bucay et al. Genes Dev 1998;12:1260–8.
8. Morony et al. J Bone Miner Res 1999;14:1478–85.
9. Capparelli et al. Cancer Res 2000;60:783–7.
10. Lacey et al. Am J Pathol 2000;157:435–48.
11. Feige et al. Cell Mol Life Sci 2000;57:1457–70.

Establishment of a vicious cycle between osteoclasts and metastatic cancer cells is critical in bone metastases

Toshiyuki Yoneda

Department of Biochemistry, Osaka University Graduate School of Dentistry, Osaka, Japan

Bone is one of the most preferential target sites of distant metastasis of cancer. As representative examples, it has long been recognized that breast and prostate cancer more frequently spread to bone than to the lung or liver. However, the mechanism by which these cancers show a predilection for spreading to bone is unclear. Bone has several unique features that are not seen in other preferential target organs of cancer metastasis, such as lung and liver. It is a calcified hard tissue, and destruction of the hard tissue is a prerequisite for metastatic cancer cells to colonize successfully in bone. Evidence accumulated in humans and experimental animals suggests that cancer cells are unable to destroy bone directly, and that osteoclasts play a key role in bone destruction during the development of bone metastases. To stimulate osteoclastic bone destruction and thereby facilitate their colonization in bone, metastatic cancer cells produce cytokines which promote osteoclastogenesis, and/or activate osteoclasts such as parathyroid hormone-related protein (PTH-rP) in a paracrine manner. Overexpression of PTH-rP cDNA in cancer cells increased bone metastases, and a neutralizing antibody to PTH-rP inhibited the development of new bone metastases and the progression of established metastases. Meanwhile, as a consequence of osteoclastic bone resorption, growth factors, including insulin-like growth factors (IGFs) and transforming growth factor b (TGFb), that are abundantly stored in the bone matrix are released, and in turn promote the survival, proliferation, and production of PTH-rP in metastatic cancer cells. The disruption of IGF signal transduction increased apoptosis in metastatic cancer cells and markedly inhibited bone metastases. Similarly, inhibition of TGFb signaling decreased PTH-rP production in cancer cells and suppressed bone metastases. Furthermore, a recent study has suggested that calcium ion ($Ca2^{+}$) released from bone following osteoclastic bone resorption also stimulates the production of PTH-rP through $Ca2^{+}$-sensing receptors on the cell membrane of metastatic cancer cells. It is therefore probable that the establishment of this vicious cycle between osteoclasts and metastatic cancer cells leads to the development and advancement of bone metastases.

Since these results suggest that osteoclasts play an important role in bone metastases, it can be reasoned that the osteoclast is a therapeutic target for suppressing bone metastases. In support of this notion, specific osteoclast inhibitor bisphosphonates (BP) are shown to suppress bone metastases effectively in cancer patients. We have also shown that in animal models, the administration of BP in a preventative or therapeutic manner significantly inhibits the development of new bone metastases or impairs the progression of established bone metastases. Importantly, we also found that BP enhanced apoptosis in osteoclasts in bone metastases and in culture. Furthermore, we observed that BP administration also increased apoptosis in metastatic cancer cells in bone. Although it is not currently known whether BP has some anticancer action, these results suggest that BP inhibits bone metastases by enhancing apoptosis in both osteoclasts and metastatic cancer cells. They also suggest that pharmacological approaches that are designed to disrupt the vicious cycle existing between osteoclasts and metastatic cancer cells are effective and beneficial for the treatment of bone metastases in cancer patients.

Regulatory mechanism of osteoblast differentiation and bone formation

Akira Yamaguchi

Department of Oral Pathology, Nagasaki University School of Dentistry, Nagasaki, Japan

Skeletal tissue is composed of various types of mesenchymal cells such as osteoblasts, chondrocytes, myoblasts, and bone marrow stromal cells including adipocytes. These cell lineages are believed to originate from common mesenchymal progenitors called pluripotent mesenchymal stem cells.

These progenitors acquire specific phenotypes depending on their maturation during differentiation. Osteoblasts express various phenotypic markers such as high alkaline phosphatase (ALP) activity, and synthesize collagenous and noncollagenous bone matrix proteins, including osteocalcin. Mature osteoblasts form mineralized bones. I here review the regulatory mechanism of osteoblast differentiation mediated by local factors such as bone morphogenetic proteins (BMPs) and hedgehogs, and the transcription factor, core-binding factor α-1 (Cbfa1).

BMPs, which belong to the TGFb-superfamily, are the most potent regulators of osteoblast differentiation among the local factors. BMPs not only stimulate osteoprogenitors to differentiate into mature osteoblasts, but also induce non-osteogenic cells, such as a certain myogenic cell, to differentiate into osteoblast lineage cells.

The hedgehog-signaling pathway mediates inductive events during development in invertebrates and vertebrates. In *Drosophila*, hedgehog signaling induces expression of decapentaplegic (dpp), which is a homologue of vertebrate BMP, in adjacent cells, whereupon dpp acts as a secondary signaling molecule to control the fate of these cells. In higher vertebrates, sonic and Indian hedgehogs are involved not only in skeletal formation during development and skeletal repair, but also in osteoblast differentiation by interacting with BMPs.

Transcription factors that determine the differentiation pathways of specific mesenchymal cell types have been identified in several cell lineages. In the case of skeletal muscles, the muscle-specific transcription factors of the MyoD family, which belong to the basic helix–loop–helix family, are necessary for determining the pathway of differentiation into the muscle lineage, and are required for the differentiation of committed myoblasts to fully differentiated myotubes. In addition, PPARg2 has been reported to play an important role in determining the differentiation pathway of adipocyte lineage cells. Cbfa1, which belongs to the runt-domain gene family, is an important transcription factor for osteoblast differentiation and bone formation. In Cbfa1-deficient embryos at E18.5, parts of the tibia, radius, and vertebrae were weakly calcified, and no calcification occurred in the skull, mandible, humerus, or femur. Histological examination showed that Cbfa1-deficient embryos completely lacked ossification. Many mRNAs related to bone matrix proteins such as osteocalcin, osteopontin, and a1(I) collagen have Cbfa1 binding sites in their promoter regions. As expected from these promoter sequences, Cbfa1 mutant mice expressed extremely low levels of osteopontin and a1(I) collagen, and had no osteocalcin in their skeletons. This indicated that the maturational arrest of osteoblasts caused the lack of bone formation in Cbfa1-deficient mice. Since it has been reported that BMP upregulates expression of Cbfa1 during osteoblast differentiation, Cbfa1 seems to be a downstream factor controlled by BMP. Thus, the intimate interactions between local factors, including BMPs and hedgehogs, and the transcription factor Cbfa1 play crucial roles in the process of osteoblast differentiation and bone formation.

References

1. Endocrinol Rev 2000;21:393–411.
2. Cell 1997;89:755–64.

Proteolysis and the skeleton in health and arthritis

A. Robin Poole

Joint Diseases Laboratory, Shriners Hospital, Montreal, Canada

Metalloproteinases (MMPs) play a critical role in skeletal development and in the maintenance of normal tissue organization, particularly that of the extracellular matrix. In arthritis, these proteinases can cause the destruction of joint tissues such as articular cartilages. This presentation will examine examples of the critical importance of normal proteolysis mediated by MMPs, and address the involvement of some of these proteinases and how pathology is generated. Emphasis will be placed on the digestion of the collagen fibril and its importance in development and disease.

References

1. Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 1997;99:1534–45.
2. Mwale F, Billinghurst RC, Ionescu M, Webber C, Alini M, Poole AR. The assembly and degradation of types II and IX collagens associated with expression of the hypertrophic phenotype. Dev Dyn 2000;218:648–62.
3. Neuhold LA, Killar L, Zhao W, Sung M-LA, Warner L, Kulik J, et al. Osteoarthritis develops in transgenic mice expressing constitutively active human collagenase-3 (MMP-13) in articular cartilage. J Clin Invest 2001.

Osteoporosis and bisphosphonates

Toshitaka Nakamura

Department of Orthopedics, University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan

In humans, marked bone loss associated with the menopause occurs in skeletal regions characterized by a high trabecular bone content. With the cessation of ovarian function, bone turnover such as resorption and formation increases. Japanese women, for example, show a reduction in lumbar trabecular bone at a mean annual rate of 5.47% during the perimenopausal period. They also loose tibial cortical bone mass at an average annual rate of 3.84%.

Bone loss is not always associated with estrogen loss. In nonhuman primates such as baboons and rhesus monkeys, ovariectomy increases the bone turnover to more than double, leading to marked lumbar bone loss. In cynomolgus monkeys, however, the stimulation of systemic bone turnover is mild, resulting in no decrease in lumbar bone. Thus,

the changes in the amount of bone mass depend on the rates of increases in bone turnover after estrogen loss.

Bone turnover takes place at the bone surfaces facing the marrow space, including trabecular, endocortical, and intracortical surfaces. Local bone turnover at these sites determines the bone loss in the skeletons, and the effect of estrogen differs in these bone surfaces. In humans, trabecular bone surfaces are apparently more sensitive to estrogen loss than are cortical bone surfaces.

Bisphosphonates regulate bone turnover by reducing osteoclastic resorption at both the trabecular and cortical bone, reducing fracture risk in the lumbar bone and femur in postmenopausal osteoporosis. The effects are contrasted with the effects of signals through estrogen receptor, which selectively reduce the fracture risk at the lumbar bone. Further, accumulated data indicate that bisphosphonates increase the duration of the bone formation period in a remodeling cycle, thus improving the mineralization of bone tissue. Thus, bisphosphonates may provide a substantially new therapeutic approach to osteoporosis and other bone diseases that are characterized by remodeling errors.

Synovial cell activation in cartilage and bone destruction

Steffen Gay · Michel Neidhart · Janine Rethage · Stefan Kuchen · Christian Seemayer · Thomas Pap · Susumu Otsu · Yukio Shigeyama · Renate E. Gay

WHO Collaborating Center for Molecular Biology and Novel Therapeutic Strategies for Rheumatic Diseases, Department of Rheumatology, University Hospital, Zürich, Switzerland

Although most of the human genome sequence has now been published, the vast majority of the sequence is not yet assigned to a given function. The new data now being generated by high-throughput sequencing, microarrays, and gene chips listing thousands of gene fragments have not yet resulted in a major breakthrough to elucidate the function of such novel gene sequences in the pathogenesis of rheumatoid arthritis (RA). Our laboratory is utilizing various molecular approaches to detect specific sequences associated with the activated phenotype of synovial fibroblasts, as well as using gene transfer to elucidate the functional role of these sequences in modulating downstream factors associated with cartilage and bone destruction in RA. In this regard, we have identified an endogenous retroviral line-1 (L-1) segment which is expressed in synovial fluid and synovial fibroblasts, especially at sites of cartilage and bone destruction. Gene transfer studies transferring a fully functional as well as a partially deleted, nonfunctional L-1 sequence into L-1-negative synovial fibroblasts, followed by establishing a substracted cDNA library, revealed all the sequences induced by the functional L-1 segment. One of the most commonly induced genes was the human stress-activated protein kinase 2-δ (SAPK2d). SAPK2 (a–d) proteins, like other mitogene-activated protein kinases (MAPK), also called MAPK (a–d), have been shown to enhance the production of matrix metalloproteinases (MMPs). Since the upregulation of MMPs, in particular MMP-1, MMP-13, and MT-MMP-1, is detected in synovial fibroblasts at sites of cartilage and bone destruction, we searched for the presence of a proton pump in these cells. Two isoforms could be detected at sites of destruction, a wild-type and a mutated form lacking one exon.

References

1. Neidhart M, Rethage J, Kuchen S, Kunzler P, Crowl RM, Billingham ME, et al. Retrotransposable L-I elements expressed in rheumatoid arthritis synovial tissue – association with genomic DNA hypomethylation and influence on gene expression. Arthritis Rheum in press.
2. Kuchen S, Gay RE, Gay S, Neidhart M. The ORF1/p40 protein of human retrotransposable L-1 elements induces stress-activated protein kinase 2 delta (SAPK2d) in rheumatoid arthritis synovial fibroblasts (RA-SF). Arthritis Rheum 2000;43:S563.
3. Otsu S, Pap T, Shigeyama Y, Aicher W, Pap G, et al. Identification of a novel splice variant of an osteoclast-like v-ATPase beta-1 subunit in activated fibroblasts. Arthritis Rheum 2000;43:S591.