[Frontiers in Bioscience 10, 54-64, January 1, 2005]

CITRULLINATED PROTEINS IN RHEUMATOID ARTHRITIS

Ryo Yamada [1], Akari Suzuki [1], Xiaotian Chang [1] and Kazuhiko Yamamoto [1, 2]

[1] Laboratory for Rheumatic Diseases, SNP Research Center, Riken, Yokohama, Japan, [2] Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

TABLE OF CONTENTS

1. ABSTRACT

Citrullinated proteins that are produced by enzymatic deimination of arginine residues in proteins by peptidylarginine deiminases (PADIs) are of particular interest in the pathogenesis of rheumatoid arthritis (RA). First, peptidylarginine deiminase type 4 (PADI4) gene, which codes one of the PADI enzyme isotypes, has a genetic variant that increases susceptibility to RA. The RA-susceptible variant of PADI4 seems to increase the risk of RA by increasing its enzymatic activity. Second, this post-translational protein modification unfolds proteins by loss of a positive charge in arginine residues, with a subsequent change in antigenicity of the self-proteins. Third, these citrullinated proteins are recognized by anti-citrullinated peptide antibodies that are the most RA-specific autoantibodies. Finally, the expression of the PADI enzyme, citrullination of proteins, and production of anti-citrullinated protein antibodies occur in synovium. These data suggest that citrullination of proteins by PADI is related to alteration of antigenicity of peptides and very closely linked to pathogenesis of RA autoimmunity.

2. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder with autoimmune features that affects 0.5% to 1.0% of the world's population. RA is characterized by the destruction of multiple joints along with multiple organ involvement. The etiology of RA is still unknown. Several causes have been proposed, including 1) genetic factors, 2) immune and inflammatory responses against microbial pathogens, 3) autoimmune responses directed against components of the synovium and cartilage, 4) autoantibodies and autoreactive T cells, 5) disordered regulation of production of proinflammatory and tissue-destructive cytokines, and 6) autonomous, tissue-invasive cells and tissues. Although the above-mentioned causes seem to interact to develop RA, it is likely that autoimmune factors play a central role in the pathogenesis RA. However, the precise autoimmune mechanism in the pathogenesis of RA is still controversial.

Cellular immune mechanisms have been implicated to have an important role in RA. First, many T cells and antigen-presenting cells are present in RA synovial tissues. (1) Second, synovial T cells are known to be activated in RA. (2) Third, T cells in RA synovial tissue are not accumulated randomly. (3) Fourth, the HLA DRB gene has been shown to be strongly associated with RA. (4) Fifth, therapy against T cells seems effective in RA. Finally, T-cell cytokines are present in RA synovium. (5) Various evidences also support the involvement of B cells in RA: For example, in RA synovium, B cells differentiate and plasma cells produce antibodies. In addition, B cells in patients with RA show oligoclonality and an autoantibody-producing subset of B cells have been seen to increase in patients with RA. (6)

Although various autoantigens were proposed as targets of pathogenic T cells and B cells in RA, none of their pathogenic mechanisms or significance has been confirmed. Recently, autoantibody recognizing citrullinated self-proteins (anti-citrullinated antibodies) were reported to be the most specific autoantibody in RA (7) and a gene that codes an enzyme producing citrullinated proteins (peptidylarginine deiminase type 4 ([PADI4 gene]) was identified to be associated with RA. (8) These findings strongly suggest that citrullinated proteins and anti-citrullinated peptide antibodies have a pathogenic role in autoimmunity in RA.

3. CITRULLINATION: POST-TRANSLATIONAL DEIMINATION OF ARGININE RESIDUES

3.1. Citrulline and arginine

Citrulline is an amino acid that is not directly

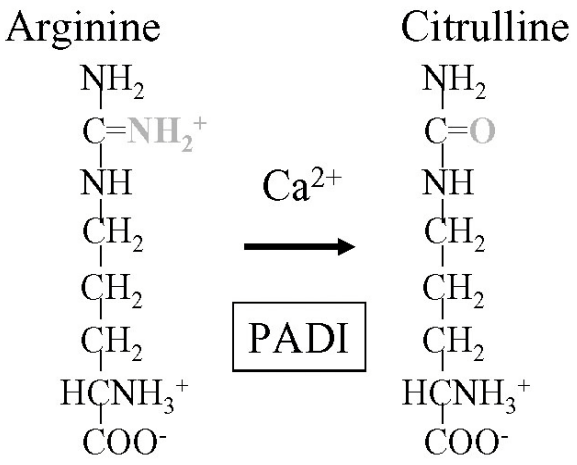


Figure 1. Enzymatic conversion of arginine to citrulline by PADI. The reaction requires high concentration of Ca^{2+}.

encoded by DNA or RNA. It is a deiminated form of arginine (Figure 1). The biggest difference between arginine and citrulline is that arginine is one of the most basic amino acids and citrulline lacks the charged feature. Citrulline is a member of the citric acid cycle, and its metabolism is tightly regulated. One abnormality in the metabolism of free citrulline is hypercitrullinemia, an innate metabolic disorder. Metabolism of protein-bound citrulline is independent from metabolism of free-form citrulline. Because no citrulline tRNA exists, all citrulline residues in proteins are the result of post-translational deimination of arginine residues. The enzyme responsible for the conversion of peptidyl arginine to peptidyl citrulline is peptidylarginine deiminase (PADI) (9).

3.2. Peptidylarginine deiminase (PADI)

PADI enzymes catalyze the conversion of arginine residues to citrulline residues in proteins. Five isotypes of PADI—PADI1, 2, 3, 4 (or sometimes called 5 for historical reasons) and 6— have been cloned from several mammals, including humans. Amino acid sequences are well conserved among the isotypes of PADI (50%-55%) and among mammals (70%-95%). All these isotypes are believed to be intracellular enzymes because none of PADI peptide sequences contain a secretory signal. They possess a Ca-binding motif, and they depend on high concentrations of Ca^{2+} for their enzymatic activity. Because the required concentration of Ca^{2+} is much higher than the cytosolic concentration, conversion of arginine residues to citrulline residues should be carried out in a microenvironment where extraordinarily high concentrations of Ca^{2+} are achieved in tightly regulated conditions or in an extracellular environment along with leakage of enzyme from dying cells. Mammalian PADIs are unable to convert free L-arginine to free L-citrulline. It appears that all PADI isotypes can deiminate many proteins *in vitro*, although some combinations of PADI isotypes and particular proteins tend to react more rapidly than others. (10) Several molecules have been reported to be natively and or experimentally citrullinated. Among the known citrullinated proteins are myelin basic protein, (11) filaggrin, (12-14) keratin, (15) histones, (16) vimentin, (17) and fibrinogen/fibrins. (18)

The biggest difference between the isotypes is their tissue-specific expression. Tissue distribution of the isotypes of PADIs varies. PADI1 is mainly expressed in the epidermis and uterus. PADI2 is expressed in neuronal tissue and macrophages as well as in many other tissues. PADI3 is expressed in hair follicles, and PADI4 is expressed mainly in white blood cells, especially in neutrophils and eosinophils. PADI4 is unique in that it possesses a putative monopartite nuclear localization signal (NLS) and is localized in the nucleus; other PADIs do not have an NLS sequence and are detected in cytosol. (9) PADI6 was most recently identified and it is expressed in oocytes. These differences in tissue distribution among PADI isotypes are expected to be related to their physiologic functions.

3.3. Citrullination: Physiologic function and effects on protein structure

Although some biological events, such as inflammation, apoptosis, trauma, and aging, increase post-translational citrullination, the precise physiologic role of citrullination is still unknown. (19-22) What is known about citrullination and its consequences is that a variety of proteins are citrullinated and subsequently changes their conformation. Because the enzymes are only active in high concentrations of Ca^{2+}, citrullination should occur outside of cells or in a tightly regulated intracellular condition. A basic group of arginine residue contributes by forming hydrogen bonds and determining secondary and tertiary protein structure (Figure 2). Citrulline lacks this polar feature of arginine. Therefore conversion of arginine residues to citrulline residues affects protein structures despite the fact that it produces only small difference in mass (~1 Da). Citrullinated proteins differ from their non-modified forms in their electrophoretic mobility because of changes in their molecular weight and charges, as well as their conformation. The biochemical changes caused by citrullination resemble denaturing proteins with detergents. (23) Citrullination is also reported to change the immunologic recognition by antibodies. This antigenic alteration of proteins by citrullination was confirmed by the finding that experimentally designed anti-citrullinated peptide antibodies and autoantibodies from sera of RA patients recognized deiminated proteins but not non-citrullinated forms. (7) More interestingly, citrullination of peptides increases peptide-MHC affinity and activates CD4+ T cells in HLA-DR4 transgenic mouse. (24) This finding supports the idea of alteration of antigenicity by citrullination and also implicates that a change in antigenicity by peptidyl citrullination has a role in the context of HLA-DR4-dependent antigen recognition. Citrullination and its effect on antigenicity are illustrated in Figure 3.

4. RA-RELATED PHENOMENA REGARDING CITRULLINATION AND PADI

4.1. Anti-citrullinated peptide antibody

Various autoantibodies have been detected in sera from patients with RA, including rheumatoid factor (RF). RF

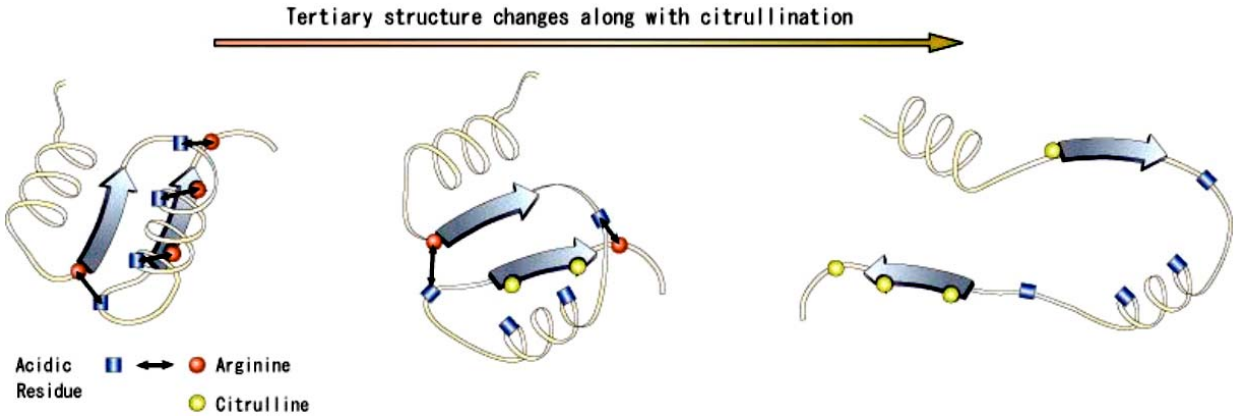


Figure 2. Deimination of arginine residues disrupts intramolecular non-covalent bonds and alters tertially structure. Basic arginine residues participate in non-covalent intra-molecular bonds. Substitution of the arginine residues loses such bonds and tertiary structure of the molecule is changed.

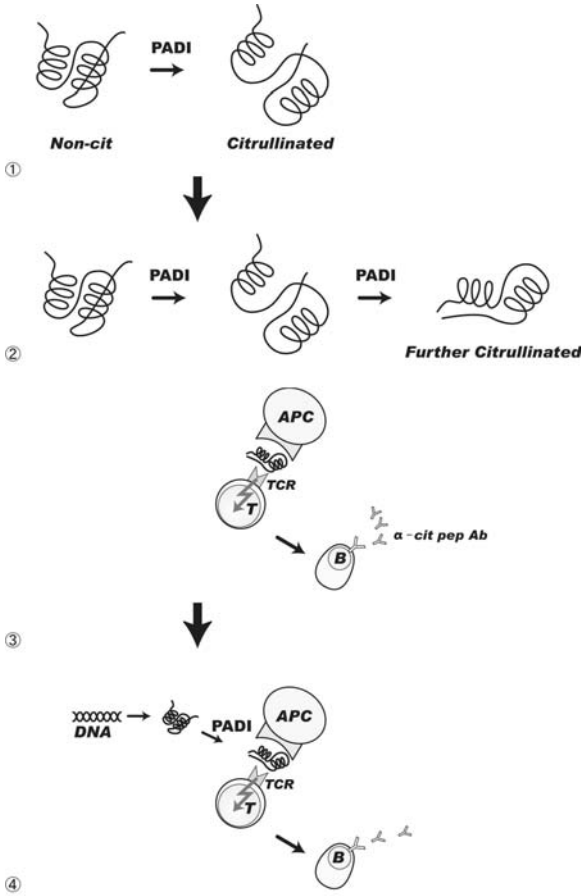


Figure 3. Citrullination of a molecule changes its tertiary structure. The change in tertiary structure seems larger when the molecule is citrullinated more (1and2). The citrullinated molecule with altered antigenicity is presented by an antigen presenting cell (APC) to T cell and the stimulated T cell signals B cell to produce anti-citrullinated peptide antibody (a-cit pep Ab) (3). In the condition where the molecule is self protein and PADI reacts on the protein, the antigenic citrullinated peptide is continuously supplied and anti-citrullinated antibody production continues (4).

recognizes the Fc portion of immunoglobulin G. Discovery of RF and its association to RA was an important step in the investigation of RA. RF is now routinely measured in clinical laboratories and its presence in serum is one of the American College of Rheumatology's classification criteria for patients with RA. (25) However, using RF levels to diagnose RA is inadequate. Its sensitivity was reported to be 75% to 90% even in very selected population with low specificity (26, 27).

Besides RF, several autoantibodies have been reported to be more specific and to have higher positive predictive value for RA. Antiperinuclear factor (28) and anti-keratin antibody (29) have a sensitivity of 43% to 52% and a specificity of 97% to 99%. (30, 31) Anti-Sa antibody was reported to have sensitivity of 27% to 50% and specificity of 99%. (32) These highly RA-specific autoantibodies have better positive predictive values due to their high specificity, and all of them have been found to recognize citrullinated peptides. (12-14, 18, 33) Based on these findings, enzyme-linked immunosorbent assay (ELISA) systems that quantitate antibodies recognizing citrullinated antigens have been developed for use in RA. One such system uses an artificial citrulline-containing peptide designed to be circular to expose as much citrulline residue as possible. This was done because investigations of the properties of citrullinated arginine residues in various peptides that were recognized by antibodies derived from RA sera revealed that more exposed residues of citrullinated arginines were better recognized. (14, 34) The diagnostic performance of anti-citrullinated peptide antibody seems to be promising based on multiple reports. (34-37) It was found that such autoantibodies not only are very specific for RA (up to 98%), but they are detected very early in the disease or even several years before the disease onset (41) and their titre tends to correlate with an erosive subtype of RA. The anti-citrullinated peptide antibodies are believed to be produced in inflamed RA antibodies is increased among synovial immunoglobulins compared to serum. Table 1 summarizes reports on sensitivity and specificity of anti-

Table 1. Sensitivity and specificity of anti-citrullinated peptide antibody assays for rheumatoid arthritis

Authors	Year of publication	Subjects	Antigen and assay method	Sensitivity	Specificity
Simon *et al.*	1993	48 cases and 56 controls	human dermal filaggrin (IB)	75%	89%
Shellekens *et al.*	1998	134 cases vs. 154 controls	CCP* (ELISA)	76%	96%
Shellekens *et al.*	2000	134 cases vs. 154 controls	CCP (ELISA)	68%	98%
Goldbach-Mansky *et al.*	2000	106 early cases vs. early non-RA arthiritides	human dermal filaggrin (ELISA)	33%	93%
Bizzaro *et al.*	2001	98 cases vs. 232 controls	CCP (ELISA)	41%	98%
Suzuki *et al.*	2003	549 cases vs. 208 controls	CCP (ELISA)	88%	89%
			human recombinant-citrullinated-filaggrin	69%	95%

CCP*: cyclic ctrullinated peptide

citrullinated antibodies.

Although RA sera recognize citrullinated auto-antigens, the auto-antigens that function as immunogens for the anti-citrullin responses in RA are unknown. Filaggrin, which was identified for the first time as a citrullinated self-peptide recognized by RA-specific sera, (12) is not an

articular component. Therefore, it seems to be recognized by anti-citrullinated peptide antibodies as a consequence of cross-reactivity. A study of synovial tissues revealed that fibrin (ogen)s in RA synovium were citrullinated, (18) and they were recognized by anti-citrullinated antibodies in RA sera. Although fibrins might be the true self-antigens that trigger a break in tolerance as a causative event of RA, further studies need to be performed. It was reported that anti-citrullinated protein antibodies were polyclonal and a restricted set of variable region genes were used by the clones. (42) Since production of antigens that are recognized by anti-citrullinated antibodies is affected by genetic variants of PADI4 gene and epitope recognition of citrullinated peptides are influenced by HLA-DR types, it seems that there are genetic predispositions to develop anti-citrullinated antibodies.

4.2. RA-susceptible variant in the PADI4 gene

Another genetic contribution to peptidyl citrullination in RA has been reported. One of the PADI genes, PADI4, was identified to be associated with RA. (8) All the genes coding isotypes of PADI enzymes are located in a single cluster. The human PADI gene cluster spans a 350kb segment in chromosome 1p36.1 (Figure 4). The PADI4 gene has two major haplotypes: one is RA-sensitive and the other is RA-non-sensitive. The two haplotypes consist of four single nucleotide polymorphisms (Figure 5). The relative risk of RA in individuals with two copies of the susceptible haplotype is 1.97 compared with individuals without a copy of the susceptible haplotype. (8) Because transcription from a susceptible haplotype is more stable than the other common haplotype of PADI4 gene, it is hypothesized that increased activity of PADI4 produces susceptibility to RA.

4.3. Citrullination and autoimmune reaction to citrullinated proteins in arthritic synovial tissue

There is evidence that citrullination of arginine residues occurs locally in RA synovium. (43, 44) PADI2 is expressed in macrophages and PADI4 in granulocytes. Both macrophages and granulocytes are present in RA synovial tissue. The expression of PADI4 was also reported in RA synovial tissue. (8) The mouse counterpart of PADI2 and PADI4 was also detected in inflammatory joints in mouse RA models. (45) Citrullinated proteins were also detected in RA synovium. One of the citrullinated proteins in RA synovium was shown to be fibrin (ogen), and it was recognized by anti-citrullinated protein antibody from RA patients. (18) Although citrullinating enzymes and citrullinated proteins are present in RA synovium, they were also detected in inflamed joints in diseases other than RA (unpublished data). Therefore citrullination and citrullinated proteins are not specific to RA synovium.

5. SUMMARY AND PERSPECTIVE

Much data exist regarding peptidyl citrulline and RA. (1) The presence of autoantibodies recognizing citrullinated self-proteins is highly specific to RA. (2) The production of anti-citrullinated peptide autoantibodies precedes the development of RA, or at least precedes the manifestation of clear symptoms of RA. (3) T cell recognition of citrulline-containing peptides is accelerated by RA-susceptible HLA-DR4 epitopes. (4) In addition, anti-citrullinated peptide antibodies are produced in inflammatory RA synovium. (5) Furthermore a genetic polymorphism that seems to increase the enzymatic activity of one of the citrullinating enzymes increases the susceptibility to RA.

Although all these data suggest that citrullination and citrullinated proteins play key roles in the pathogenesis of RA, they do not explain all of the relationship between citrulline and RA. First, neither the expression of PADI nor the presence of citrullinated proteins in the synovium is restricted to RA. Second, the fact that the production of anti-citrullinated peptide antibodies precedes disease onset contradicts the simple hypothesis that citrullination and/or anti-citrullinated peptide antibodies are pathogenic on their own.

Figure 6 describes the processes that are present or absent in symptomatic RA, non-RA arthritis, and the preclinical period of RA. When inflammation is established in RA joints, inflammatory cells accumulate in the synovium and inflammatory cytokines are produced. The PADI enzyme is activated with an appropriate Ca^{2+} concentration.

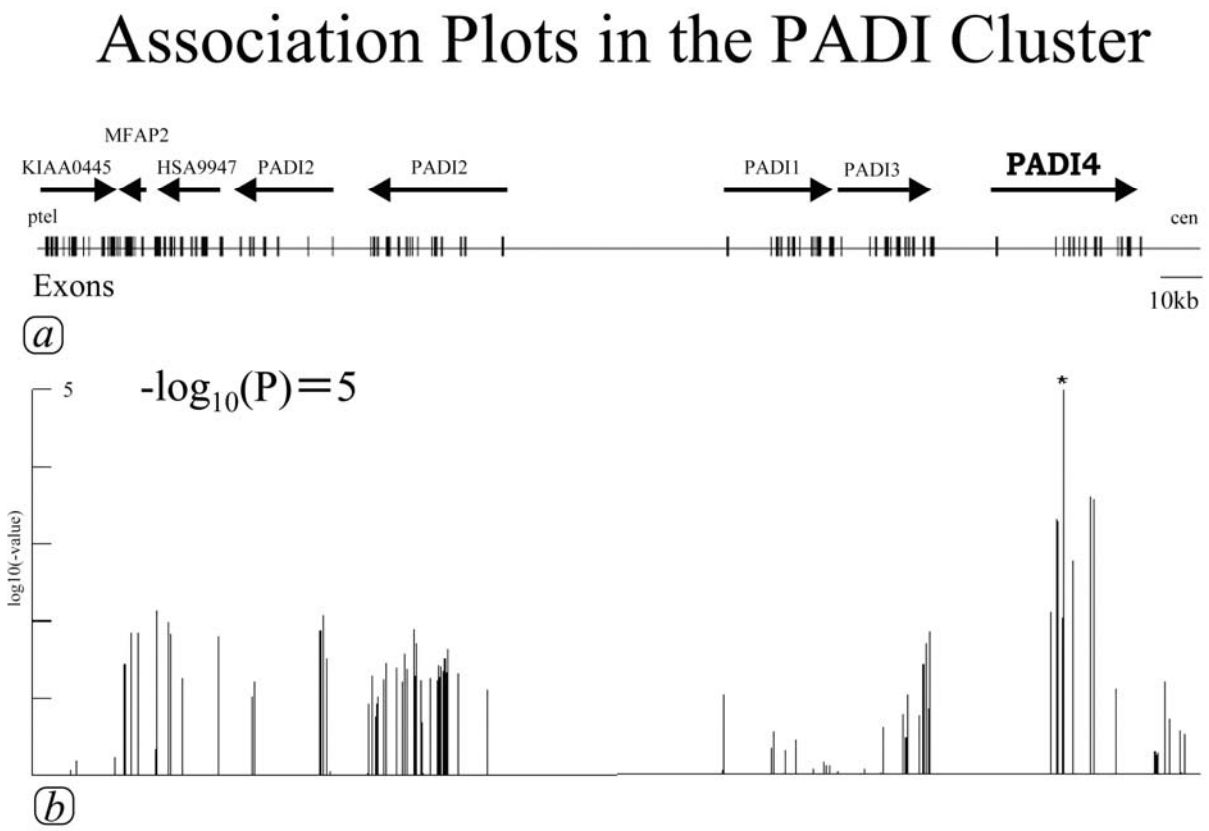


Figure 4. Result of linkage disequilibrium mapping of the PADI-cluster region. a indicates distribution of genes and SNPs. The vertical axis of b represents negative value of logarithm of P in case-control association test on each SNP. PADI1, 2, 3 and 4 genes cluster and association with RA was detected in PADI4 gene.

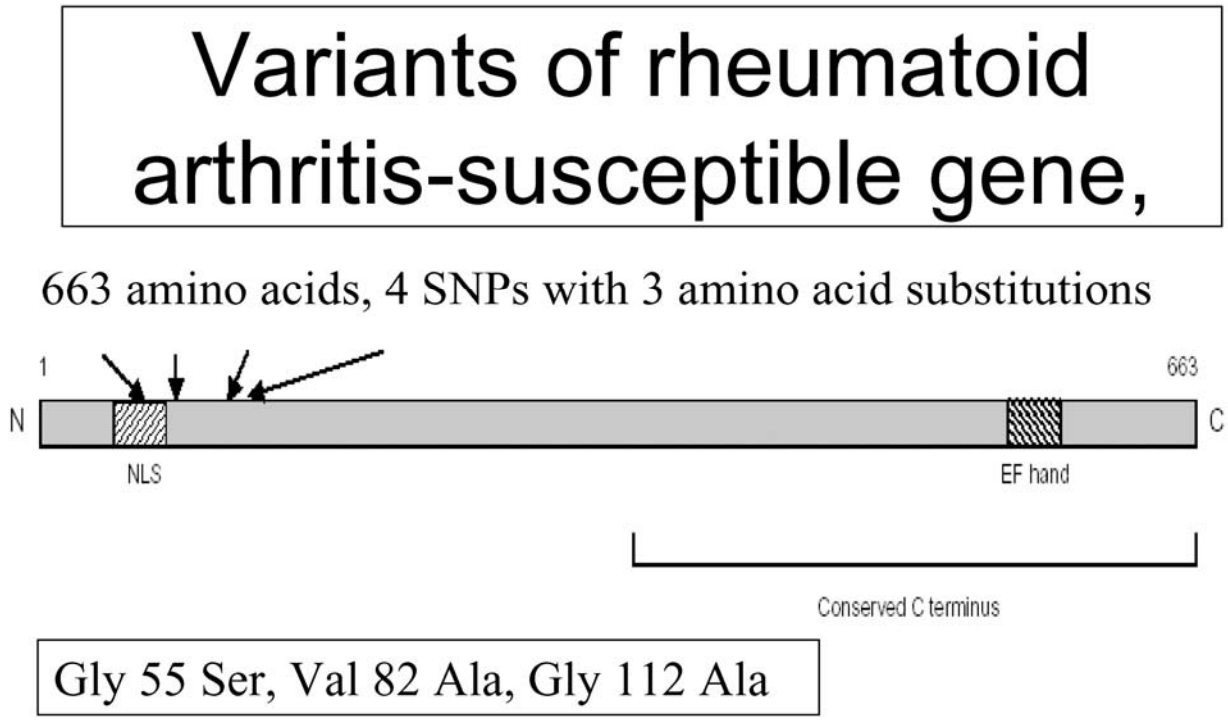
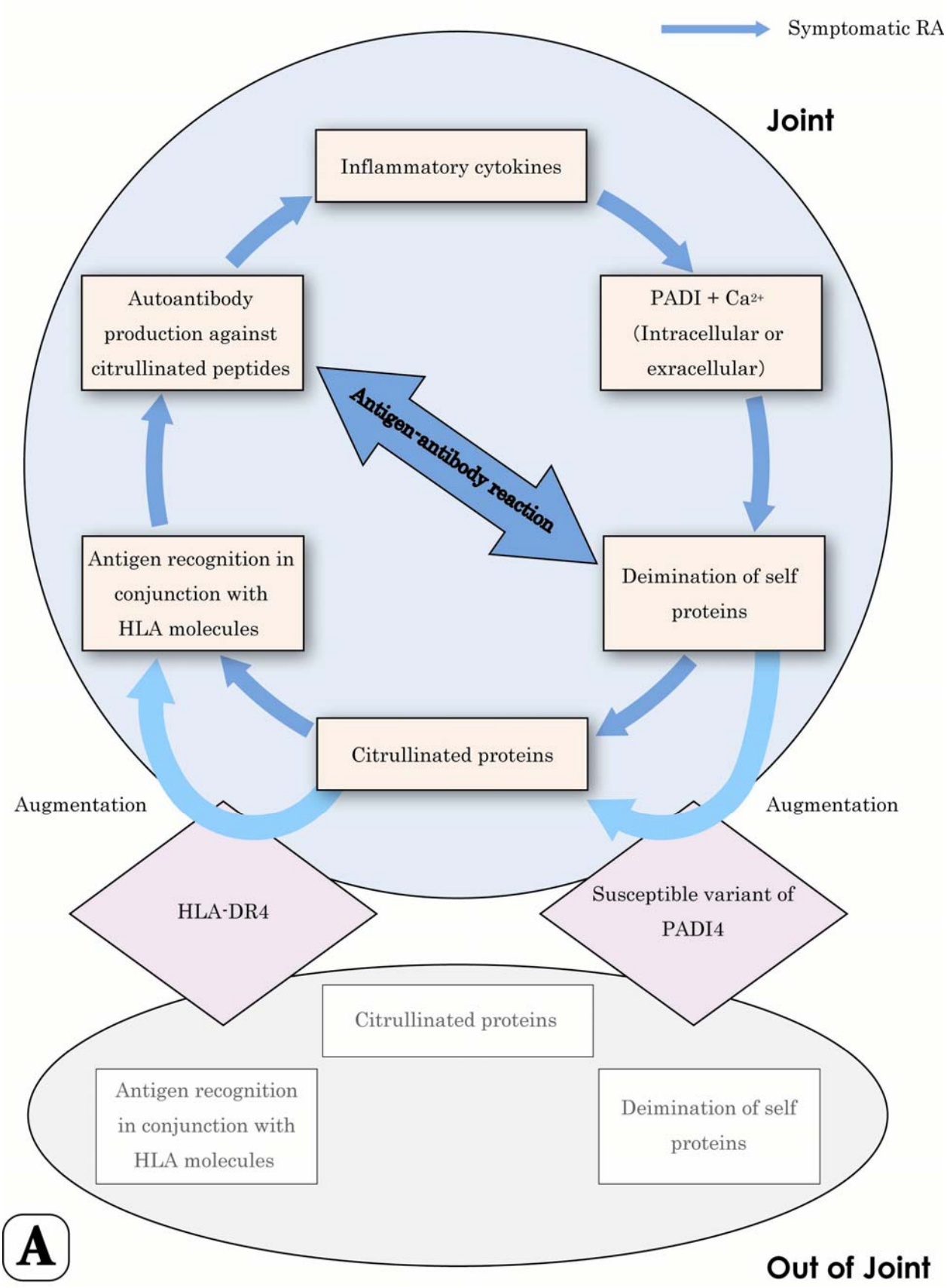
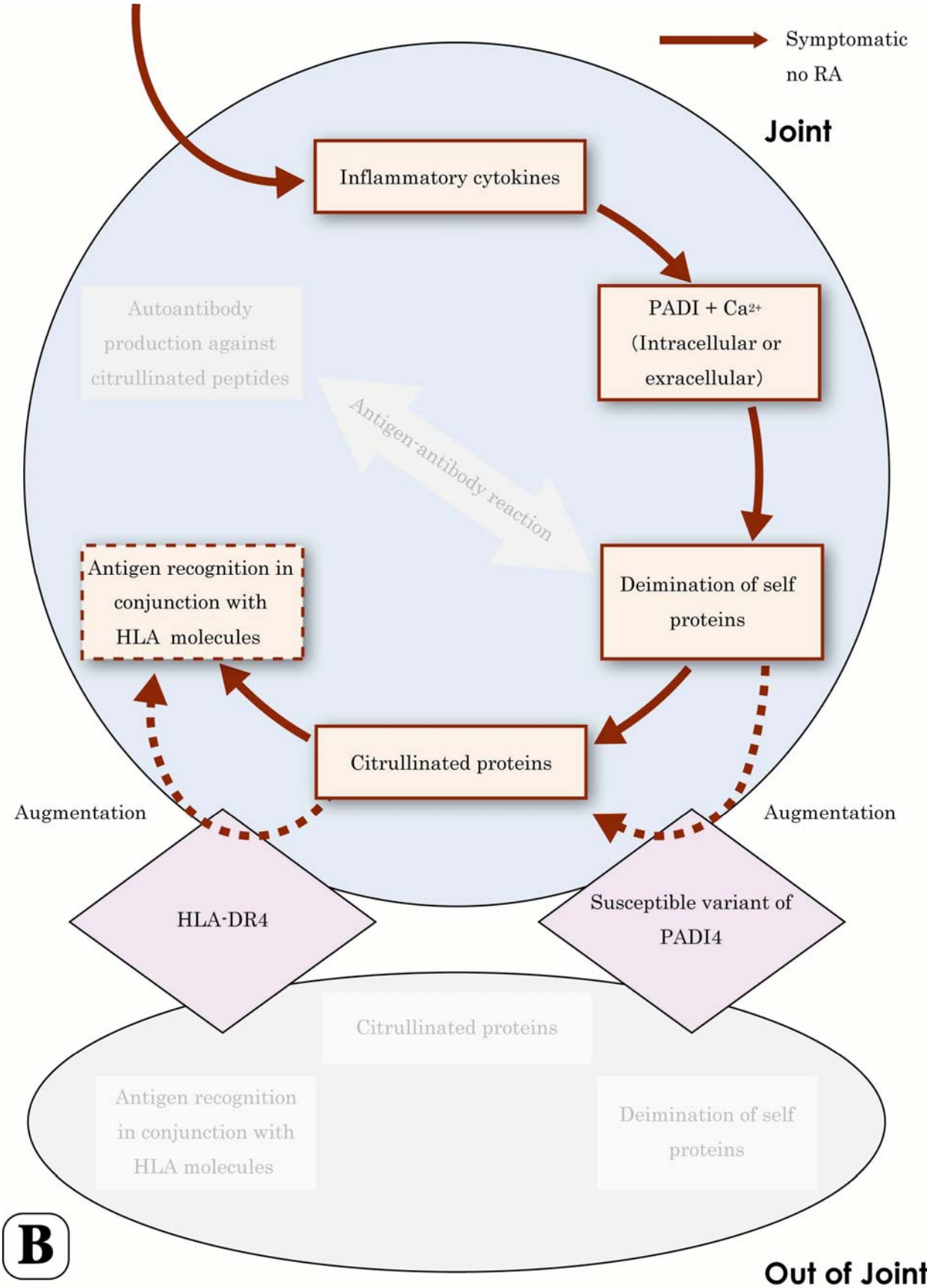


Figure 5. PADI4 molecule is consisted of 663 amino acids. There is a nuclear leading sequence and EF-hand (calcium-binding motif) and its C-terminal is conserved among PADI isotypes. There are two variants in PADI4 gene with 4 SNPs and 3 amino acid substitutions.


Symptomatic RA
Joint
Inflammatory cytokines
PADI + Ca2+
(Intracellular or
exracellular)
Deimination of self
proteins
Citrullinated proteins
Antigen recognition in
conjunction with
HLA molecules
Autoantibody
production against
citrullinated peptides
Antigen-antibody reaction
Augmentation
Augmentation
HLA-DR4
Susceptible variant of
PADI4
Citrullinated proteins
Antigen recognition
in conjunction with
HLA molecules
Deimination of self
proteins
A
Out of Joint

Pivotal role of citrullinated proteins in pathogenesis of rheumatoid arthritis



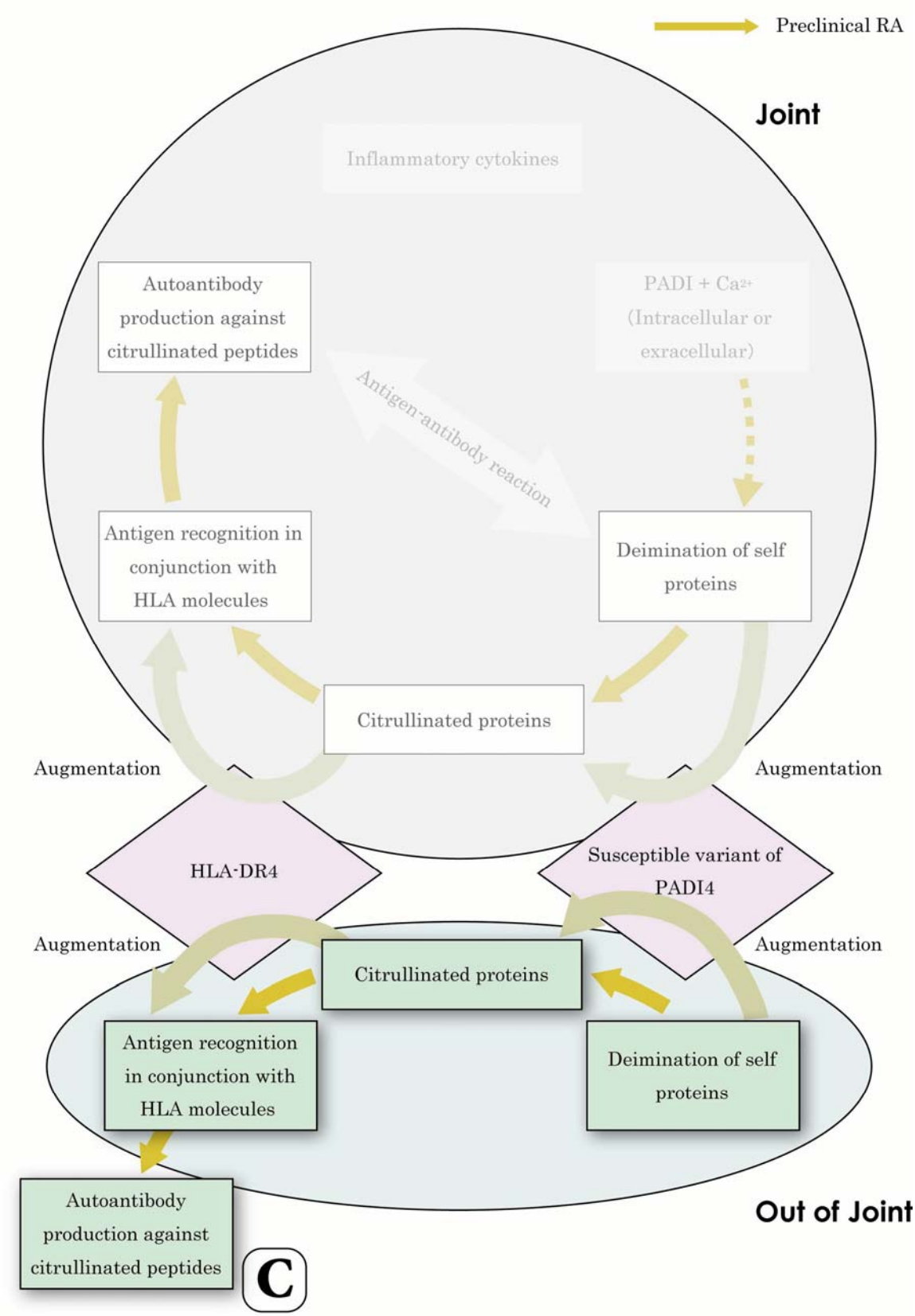


Figure 6. Citrullination, production of anti-citrullinated peptide antibodies, and inflammation are illustrated for inflammatory RA joint (A), inflammatory non-RA joint (B), and pre-clinical RA condition (C). Descriptions of each panel are in the main text.

Deimination of arginine residues occurs and citrullinated proteins are produced in synovial tissue. Because anti-citrullinated peptide antibodies and citrullinated peptide-reactive lymphocytes are present in inflammatory RA synovium, an autoimmune reaction against the citrullinated proteins occurs, and subsequently inflammation is accelerated. Thus, a cycle of reactions is established. It appears that ongoing chronic inflammation of RA could be explained by this circular reaction. In this scenario, RA-susceptible HLA-DR alleles may promote the process that citrulline-containing peptides activate autoreactive lymphocytes, but they are not indispensable. In the same way, genetic predisposition to higher expression of PADI4 can work to promote the production of citrullinated proteins, but the step can be still active without the variant. On the other hand, in non-RA arthritis, inflammatory cells, and cytokines accumulate in the joints and subsequently the PADI enzyme is activated and citrullinated proteins are produced. The citrullinated proteins might be presented to T cells by antigen-presenting cells, but somehow, autoimmunity against citrullinated peptides does not develop. In this case, a circular reaction does not occur. Thus, some other factor(s) that keeps arthritis continuous must be present.

Because anti-citrullinated peptide antibodies are detected in sera several years before the diagnosis or even before the initial manifestation of RA symptoms, production of citrullinated proteins and anti-citrullinated peptide antibodies should occur under different conditions from symptomatic RA. What differentiates symptomatic RA from the preclinical condition of RA is presence of inflammation and possibly a local reaction between citrullinated proteins and anti-citrullinated peptide antibodies in the inflamed synovium. What is common between preclinical RA and symptomatic RA is presence of anti-citrullinated antibodies in the serum. In addition, genetic predisposition to RA (HLA-DR and PADI4) must be common in preclinical and clinical RA. In the preclinical phase of RA, synovial inflammation is absent or negligible. Therefore autoimmunity against citrullinated peptides should be established in joints without inflammation or only with negligible inflammation, or the autoimmunity should be started somewhere else such as lymph node or bone marrow.

Although it is very likely that citrullination has an important role in RA pathogenesis, several questions still need to be answered: (1) How is the immunologic tolerance for citrullinated and non-citrullinated forms of self-proteins achieved and maintained? (2) What is the physiologic role of citrullination? (3) What is the difference between physiologic citrullination and pathogenic citrullination? (4) What is the initial citrullinated protein that triggers the citrulline-related autoimmuity and where does it start? (5) Is there any pathogenic citrullinated protein that induces RA? If there is, what is it? (6) Because joints are the primary loci of disease activity of RA, citrulline-related autoimmune reactions should have some joint-specific components. What are they? Investigations to answer these questions will provide us with a better understanding of RA.

6. ACKNOWLEDGMENTS

We thank all the members of Laboratory for Rheumatic Diseases, SRC, RIKEN, particularly Ms. K. Komakine for drawing illustrations.

7. REFERENCES

1. Athanasou, N. A. and J. Quinn: Immunocytochemical analysis of human synovial lining cells: phenotypic relation to other marrow derived cells. *Ann Rheum Dis* 50, 311-315 (1991)

2. Denning, S. M., P. T. Le, K. H. Singer and B. F. Haynes: Antibodies against the CD44 p80, lymphocyte homing receptor molecule augment human peripheral blood T cell activation. *J Immunol* 144, 7-15 (1990)

3. Veale, D. J. and C. Maple: Cell adhesion molecules in rheumatoid arthritis. *Drugs Aging* 9, 87-92 (1996)

4. Nepom, G. T: Major histocompatibility complex-directed susceptibility to rheumatoid arthritis. *Adv Immunol* 68, 315-332 (1998)

5. Tagaya, Y., R. N. Bamford, A. P. DeFilippis and T. A. Waldmann: IL-15, a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity* 4, 329-336 (1996)

6. McGee, B, R. E. Small and R. Singh: B lymphocytic clonal expansion in rheumatoid arthritis. *J Rheumatol* 23, 36-43 (1996)

7. van Boekel, M. A., E. R. Vossenaar, F. H. van den Hoogen, W. J. van Venrooij: Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 4, 87-93 (2002)

8. Suzuki, A., R. Yamada and X. Chang: Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* (2003)

9. Vossenaar, E. R., A. J. Zendman, W. J. van Venrooij and G. J. Pruijn: PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioessays* 25, 1106-1118 (2003)

10. Senshu, T., K. Akiyama, A. Ishigami, K. Nomura: Studies on specificity of peptidylarginine deiminase reactions using an immunochemical probe that recognizes an enzymatically deiminated partial sequence of mouse keratin K1. *J Dermatol Sci* 21, 113-126 (1999)

11. Zhou, S. R., J. N. Whitaker, D. D. Wood, M. A. Moscarello: Immunological analysis of the amino terminal and the C8 isomer of human myelin basic protein. *J*

Neuroimmunol 46, 91-96 (1993)

12. Simon, M., E. Girbal, M. Sebbag: The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis. *J Clin Invest* 92, 1387-1393 (1993)

13. Girbal-Neuhauser, E, J. J. Durieux and M. Arnaud: The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. *J Immunol* 162, 585-594 (1999)

14. Schellekens, G. A., B. A. de Jong, F. H. van den Hoogen, L. B. van de Putte and W. J. van Venrooij: Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 101, 273-281 (1998)

15. Senshu, T., S. Kan, H. Ogawa, M. Manabe, H. Asaga: Preferential deimination of keratin K1 and filaggrin during the terminal differentiation of human epidermis. *Biochem Biophys Res Commun* 225, 712-719 (1996)

16. Hagiwara, T., K. Nakashima, H. Hirano, T. Senshu, M. Yamada: Deimination of arginine residues in nucleophosmin/B23 and histones in HL-60 granulocytes. *Biochem Biophys Res Commun* 290, 979-983 (2002)

17. Asaga, H., M. Yamada, T. Senshu: Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. Biochem Biophys Res Commun 243, 641-646 (1998)

18. Masson-Bessiere, C., M. Sebbag, E. Girbal-Neuhauser: The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. *J Immunol* 166, 4177-4184 (2001)

19. van Stipdonk, M. J., A. A. Willems, S. Amor: T cells discriminate between differentially phosphorylated forms of alphaB-crystallin, a major central nervous system myelin antigen. *Int Immunol* 10, 943-950 (1998)

20. Rathmell, J. C. and C. B. Thompson: The central effectors of cell death in the immune system. *Annu Rev Immunol* 17, 781-828 (1999)

21. Piacentini, M. and V. Colizzi: Tissue transglutaminase: apoptosis versus autoimmunity. *Immunol Today* 20, 130-134 (1999)

22. Hershko, A. and A. Ciechanover: The ubiquitin system. *Annu Rev Biochem* 67, 425-479 (1998)

23. Tarcsa, E., L. N. Marekov, G. Mei: Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. *J Biol Chem* 271, 30709-30716 (1996)

24. Hill, J. A., S. Southwood, A. Sette: Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J Immunol* 171, 538-541 (2003)

25. Arnett, F. C., S. M. Edworthy, D. A. Bloch: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31, 315-324 (1988)

26. Wolfe, F., M. A. Cathey, F. K. Roberts: The latex test revisited. Rheumatoid factor testing in 8,287 rheumatic disease patients. *Arthritis Rheum* 34, 951-960 (1991)

27. Shmerling, R. H., T. L. Delbanco: The rheumatoid factor: an analysis of clinical utility. *Am J Med* 91, 528-534 (1991)

28. Sondag-Tschroots, I. R., C. Aaij, J. W. Smit, T. E. Feltkamp: The antiperinuclear factor. 1. The diagnostic significance of the antiperinuclear factor for rheumatoid arthritis. *Ann Rheum Dis* 38, 248-251 (1979)

29. Young, B. J., R. K. Mallya, R. D. Leslie, C. J. Clark, T. J. Hamblin: Anti-keratin antibodies in rheumatoid arthritis. *Br Med J* 2, 97-99 (1979)

30. Vincent, C., F. de Keyser, C. Masson-Bessiere: Anti-perinuclear factor compared with the so called "antikeratin" antibodies and antibodies to human epidermis filaggrin, in the diagnosis of arthritides. *Ann Rheum Dis* 58, 42-48 (1999)

31. Vincent, C., G. Serre and F. Lapeyre: High diagnostic value in rheumatoid arthritis of antibodies to the stratum corneum of rat oesophagus epithelium, so-called 'antikeratin antibodies'. *Ann Rheum Dis* 48, 712-722 (1989)

32. Despres, N., G. Boire, F. J. Lopez-Longo and H. A. Menard: The Sa system: a novel antigen-antibody system specific for rheumatoid arthritis. *J Rheumatol* 21, 1027-1033 (1994)

33. Senshu, T., K. Akiyama, S. Kan: Detection of deiminated proteins in rat skin: probing with a monospecific antibody after modification of citrulline residues. *J Invest Dermatol* 105, 163-169 (1995)

34. Schellekens, G. A., H. Visser, B. A. de Jong: The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 43, 155-163 (2000)

35. Goldbach-Mansky, R, J. Lee and A. McCoy: Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. *Arthritis Res* 2, 236-243 (2000)

36. Bizzaro, N., G. Mazzanti, E. Tonutti, D. Villalta, R. Tozzoli: Diagnostic accuracy of the anti-citrulline antibody

assay for rheumatoid arthritis. *Clin Chem* 47, 1089-1093 (2001)

37. Bas, S., T. V. Perneger and M. Seitz: Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factors. *Rheumatology (Oxford)* 41, 809-814 (2002)

38. Suzuki, K., T. Sawada, A. Murakami: High diagnostic performance of ELISA detection of antibodies to citrullinated antigens in rheumatoid arthritis. *Scand J Rheumatol* 32, 197-204 (2003)

39. Nogueira, L., M. Sebbag, C. Vincent: Performance of two ELISAs for antifilaggrin autoantibodies, using either affinity purified or deiminated recombinant human filaggrin, in the diagnosis of rheumatoid arthritis. *Ann Rheum Dis* 60, 882-887 (2001)

40. Vincent, C., L. Nogueira, M. Sebbag: Detection of antibodies to deiminated recombinant rat filaggrin by enzyme-linked immunosorbent assay: a highly effective test for the diagnosis of rheumatoid arthritis. *Arthritis Rheum* 46, 2051-2058 (2002)

41. Rantapaa-Dahlqvist, S., B. A. de Jong, E. Berglin: Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 48, 2741-2749 (2003)

42. Raats, J. M., E. M. Wijnen, G. J. Pruijn, F. H. van den Hoogen, W. J. van Venrooij: Recombinant human monoclonal autoantibodies specific for citrulline-containing peptides from phage display libraries derived from patients with rheumatoid arthritis. *J Rheumatol* 30, 1696-1711 (2003)

43. Masson-Bessiere, C., M. Sebbag, J. J. Durieux: In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. *Clin Exp Immunol* 119, 544-552 (2000)

44. Reparon-Schuijt, C. C., W. J. van Esch, C. van Kooten: Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. *Arthritis Rheum* 44, 41-47 (2001)

45. Vossenaar, E. R., S. Nijenhuis, M. M. Helsen: Citrullination of synovial proteins in murine models of rheumatoid arthritis. *Arthritis Rheum* 48, 2489-2500 (2003)

46. Wood, D. D., J. M. Bilbao, P. O'Connors, M. A. Moscarello: Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. *Ann Neurol* 40, 18-24 (1996)

47. Ishida-Yamamoto, A., T. Senshu, H. Takahashi: Decreased deiminated keratin K1 in psoriatic hyperproliferative epidermis. *J Invest Dermatol* 114, 701-705 (2000)

Key Words: Rheumatoid Arthritis, PADI4, Citrulline, Anti-Citrullinated Peptide Antibody, Review

Send correspondence to: Ryo Yamada, M.D., Ph.D., 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045, Kanagawa, Japan, Tel: +81-45-503-9569, Fax: +81-45-503-9590, E-mail: ryamada@src.riken.go.jp

http://www.bioscience.org/current/vol10.htm