Toward a Non-Empirical Treatment for Rheumatoid Arthritis Based on Its Molecular Pathogenesis

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Rheumatoid arthritis (RA) is a chronic, disabling disease that affects individuals during the productive years of their lives. Modern treatment for RA includes the so-called "biologic" therapy, which is based on recombinant proteins that modify the biologic processes. These agents have potent therapeutic effects and different mechanisms of action. Nevertheless, therapeutic failure still prevails. Treatment that prevents disability in RA must be started in an early manner, before the development of complications and, ideally, with a minimum possibility of therapeutic failure. As yet, there are no clinical or laboratory criteria to identify those patients with a higher probability of responding to particular types of therapy, delaying control of RA ad affecting the prevention of incapacity. Research into gene diversity through single-nucleotide polymorphisms (SNPs) by means of microarray systems, allows the detailed analysis of gene factors associated to a given disease. SNPs have been recently applied to the study of RA, where the major polymorphisms associated to RA occur primarily in genes that code for proteins related to the initiation of an immune response and/or the control of cellular activity in the immune system, in addition to genes related to tissue repair.
The specific meaning of these findings is in its initial stages of research. On the other hand, proteomics relate to the analysis of protein expression profiles at multiple levels. Both types of studies will contribute to the knowledge of patterns of gene expression in RA compared to the general population, and will allow an understanding of the pathogenesis of RA. Moreover, proteomic and genomic profiles can be employed to designs probes that identify individuals with the risk of developing RA, individually predict the response to different therapeutic modalities (pharmacogenomics) and for the follow-up of the biologic response to therapy.

Key words: Rheumatoid arthritis. Pathogenesis. Genomics. Proteomics. Treatment.

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Prevalencia de enfermedad vascular aterosclerótica en pacientes cubanos con lupus eritematoso sistémico

La artritis reumatoide (AR) es una enfermedad crónica e incapacitante que afecta a individuos en etapas productivas de la vida. El tratamiento moderno de la AR incluye la denominada terapia "biológica" basada en proteínas recombinantes, modificadoras de procesos biológicos, con efectos terapéuticos potentes y diferentes mecanismos de acción, pese a lo cual persisten fracasos terapéuticos.
Un tratamiento que prevenga la discapacidad en AR debe instituirse en forma temprana, antes del desarrollo de secuelas, e idealmente con mínima posibilidad de fracaso terapéutico. No existen criterios clínicos o de laboratorio que identifiquen a pacientes con mayor probabilidad de respuesta a distintas formas de terapia, lo que retarda el control de la AR y afecta a la prevención de discapacidad. El estudio de la diversidad genética, por medio de polimorfismos de una sola base (SNP) con sistemas de microarreglos (MA), permite el análisis detallado de los factores genéticos asociados a una enfermedad, lo cual empieza a utilizarse en AR. Los polimorfismos con mayor asociación con AR ocurren primordialmente en genes que codifican proteínas relacionadas con el inicio de la respuesta inmunitaria y/o con el control de la actividad celular, además de genes relacionados con la reparación tisular. El significado específico de esto apenas empieza a estudiarse. Por otro lado, la proteómica estudia los perfiles de expresión proteínica en cualquier individuo a múltiples niveles.
Ambos tipos de estudios ayudarían a conocer los patrones de expresión génica en AR comparados con la población

general. Además de ayudar a conocer la patogenia de la AR, los perfiles proteómicos y genómicos pueden utilizarse para diseñar sondas que identifiquen a individuos con riesgo de desarrollar AR, predigan en forma individualizada la respuesta a distintos esquemas terapéuticos y que permitan seguir la respuesta biológica a la terapia.

Palabras clave: Artritis reumatoide. Patogenia. Genómica. Proteómica. Tratamiento.

Introduction

In spite of the great therapeutic advances represented by the introduction of the so-called biologic agents in the treatment of rheumatoid arthritis (RA), we still should not be satisfied because, probably, we haven't even reached the midway point and what has been achieved up until now, although good, is incomplete and treatment is still, in a large part, empirical. This review has the objective of updating in a critical manner the knowledge of RA, especially its molecular pathogenesis, in order to understand the basis of current therapies and look for new, more effective treatments, targeted to potential subgroups of RA. In the first part we perform a summary of the current state of knowledge in RA therapy, followed by a review of the genetics of RA and, finally, an essay on the possible role of the different genes implicated in the susceptibility to RA in its pathogenesis in order to open the field for proposing new therapeutic targets.

Current State of Knowledge and Treatment of RA

It seems redundant to state that RA is the inflammatory illness with the largest impact in rheumatology. If its prevalence is 0.3% to 0.5%,[1-6] considering 20 to 64 year-old adults (54.5 million according to INEGI-2005), the estimated number of RA patients in Mexico would be 169 000 to 273 000, while in Spain, the 2006 census revealed a population of 44.7 millions inhabitants, with 28.6 million adults ranging from 20-64 years of age (INE); considering a prevalence of 0.5% in adults,[2,7] there would be 143 319 cases. In general, it is accepted that practically all of the RA patients referred to the rheumatologist require treatment with disease-modifying anti-rheumatic drugs (DMARD), though this might not represent all RA cases. Faced with the difficulty of preventing a disease with an obscure etiology, the objective of RA treatment is still to induce remission of activity, something currently unattainable in the majority of cases. Therefore, the alternative is to obtain the best control possible over activity, symptomatic relief, recover quality of life and functional capacity for daily living, and job related activities, as well as to prevent mortality. For this it is necessary to stop or delay structural damage, something that is done by modifying the biologic tissue damage process of RA. DMARDs are agents with the capacity to modify the biology and prognosis of RA, of which methotrexate (MTX) in dosages reaching up to 20 mg a week is still the standard treatment to the point where it is considered the baseline treatment to accompany the treatment with biologic agents.[8-16] Other DMARDs that have shown usefulness in the treatment of RA are sulphasalazine and cloroquine, especially when combined with MTX, and leflunomide with or without MTX. Gold salts and D-penicillamine have become less employed. A common denominator of these medications is that the basis of their therapeutic effect in RA is unknown, in spite of having knowledge of their pharmacologic mechanism, except in the cases of cyclosporine A and FK506,[17-19] which act by inhibiting 2 different molecules necessary for the activation of calcineurin, a phosphatase which is needed for the activation of T lymphocyte nuclear factor (NFAT), necessary for cell activation. An additional possible DMARD is rapamicine, which inhibits cell activation through another pathway, but which has not been evaluated systematically in RA (Figure 1).

Among the biologic DMARDs, therapy with anti-TNF antibodies was the first one to show therapeutic success, after several failed attempts with anti-CD4, antiICAM1, and other antibodies, which did not go past the first experimental stages, in spite of the fact that their use was based on the supposed pathogenic basis of RA. It is possible that the failure of these treatments, especially anti-CD4, is due to the fact that they were employed in patients with advanced RA and we may never get to know if its use in RA would have meant something else. In addition, it is extremely important to adequately justify its use in order to avoid catastrophic events such as the cytokine storm that occurred in healthy volunteers treated with an anti-CD28 agonist antibody.[20]

Taking all of this into account, anti-TNF therapy, including etanercept (a soluble receptor of TNF) and the anti-TNF monoclonal antibodies infliximab and adalimumab, has a variable efficacy. For example, with infliximab there was a clinical response of 51.8% versus 17% in controls at 6 months (P<.001), but only a few achieved remission.[15,21,22] With etanercept as monotherapy (25 mg 2 times a week), 59% of the patients obtained an improvement of ACR20 at 6 months, versus 11% of controls; 40% improved in ACR50 and 25%, in ACR70. Etanercept associated to MTX is effective in the short and long terms.[23-29] With adalimumab plus MTX, 62% of the patients achieved ACR50 after a year versus 46% in the group receiving only MTX. At 2 years, 59% of the patients undergoing combined therapy achieved ACR50, compared to 43% of the MTX group.[27,30-32] These results,

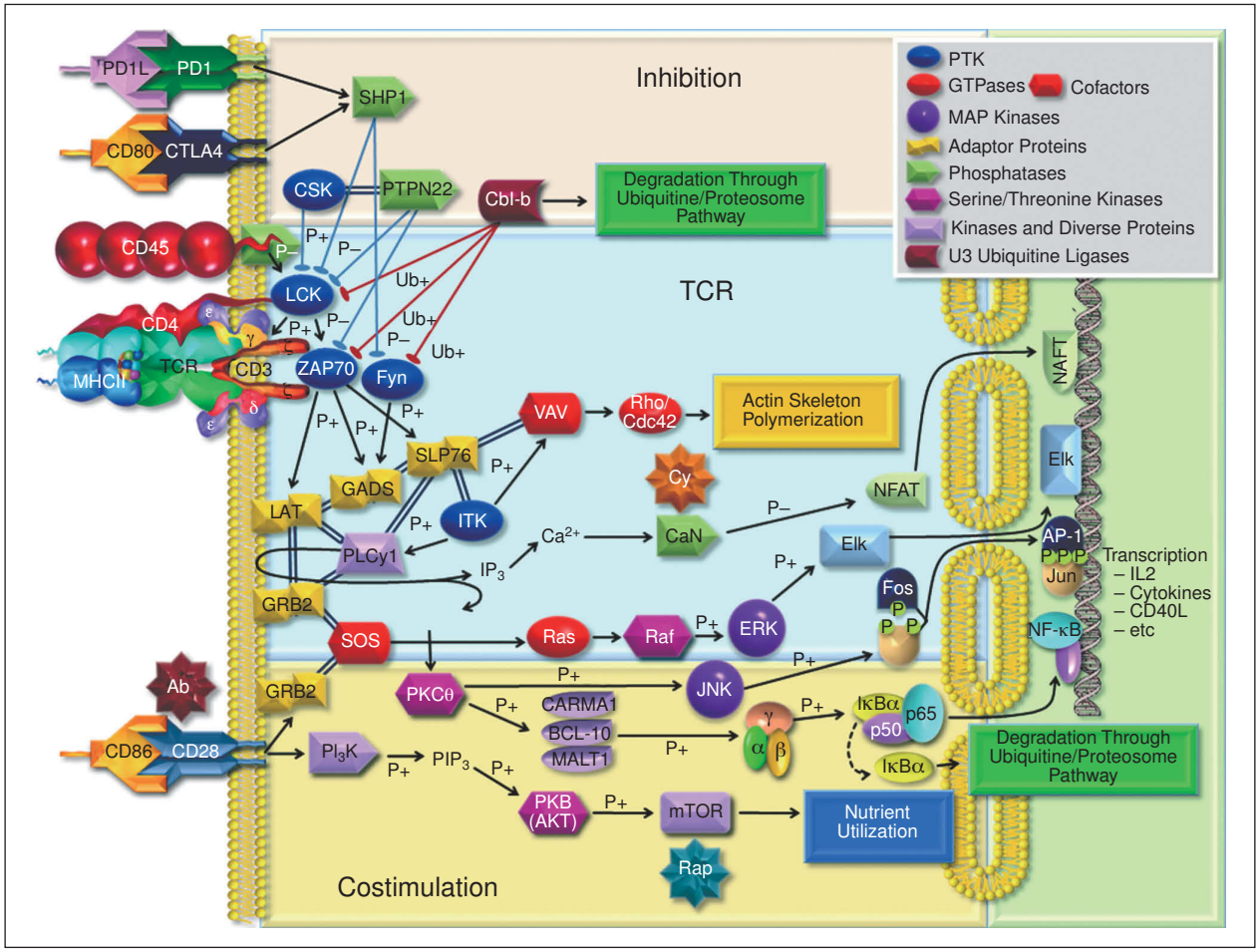

Figure 1. *Signal transduction pathways in T lymphocytes. Round pointed arrows indicate inhibition, blue when due to phosphorylation (P+) – dephosphorylation (P–). Red when due to degradation due to ubiquitine (Ub+). Active sites of abatacept (Ab), cyclosporine (Cy), and rapamicine (Rap).*

which are only examples in a vast sea of literature, show that, in spite of being effective, anti-TNF rarely induce remission. A notable effect of anti-TNF treatment is its capacity to reduce the progression of structural damage, evidenced by a lesser number of erosions and a reduced loss of cartilage, even in patients with no symptomatic improvement.

The most important side effect of anti-TNF is the development of infection (almost always endogenous reinfection) due to *Mycobacterium tuberculosis*, which is its main limitation (not a contraindication), but that, as quite an unexpected finding, helped us know that one of the functions of TNF is to prevent the dissemination of intracellular agents possibly because it is necessary for the formation of granulomas.

Rituximab is an antibody targeted against the CD20 molecule, expressed by B lymphocytes since its precursor stages in the bone marrow to before achieving plasma cell status.[33,34] Rituximab, which eliminates B lymphocytes apparently by inducing their apoptosis, has been successfully used in the treatment of non-Hodgkin's lymphoma of a B cell lineage[35,36] and seems to be effective in a relatively low number of patients with RA, in which the improvement can be noticeable, with 13% remission after the second cycle of administration.[10,37] Given that the pathogenic role of B lymphocytes in RA is unclear (reviewed by Díaz-González et al[38]), the basis of the therapeutic effects of rituximab in RA are unknown.

A third effective agent in RA is the chimerical protein abatacept, formed by the receptor CTLA-4 (expressed by activated T lymphocytes) and the Fc fraction of human IgG1. Abatacept works as an antagonist upon binding to CD86 and CD80, inhibiting their binding to their ligand CD28,[39-44] therefore preventing T lymphocyte activation at the beginning of the immune response due to competitive inhibition.[45,46] In RA, ACR20 response at 12 weeks is 53%; ACR50, 51%; and ACR70, 28%; with

abatacept plus MTX, compared with placebo and MTX, 41%, 35%, and 5%, respectively.
In conclusion, al available biologic DMARDs are effective and suppress activity, and although clinically do not seem very superior to MTX alone or in combination with cloroquine and/or sulphasalazine,[47-49] biologics delay the progression of the radiologic lesions in RA resistant to conventional treatment. Nonetheless, there are many patients who do not respond to one or more biologic agents, especially if success is defined as the induction of remission.
The fact that not all patients respond to the same treatment indicates that the pathogenesis of RA, as a complex disease, is heterogeneous, something that concurs with the variety of genetic factors associated to it. The response to one treatment or the other could depend on the genetic mosaic of each patient, reflecting pathogenic differences between them. Currently we do not know if a patient resistant to a biologic DMARD could respond to another one or even to a combination of drugs. It is essential to have rational individual treatments targeting crucial pathways of the molecular pathogenesis of RA.

The Importance of Knowing the Genetics of RA

Although many aspects of its pathogeny are still obscure, the origin of inflammation in RA is autoimmune. There is a good deal of evidence[64-69,77] that supports the participation of genetic factors in the susceptibility to RA, which interact with environmental factors for the development of disease. RA can present itself as multiple cases in the same family and susceptibility to develop it is inherited as a multigenic way, though the direct significance of each one of the associated polymorphisms, including the most studied, is still not completely understood.
For example, some genetic variants associated to RA occur in the part of the gene that directly encodes the protein. In some cases it is very likely that the mechanism implicated is an alteration in the function of the protein, be it a loss or a gain. However, other variants occur in non-codifying regions of the genome, which can be of several types:

1. Polymorphisms in the same gene:

– In regulating regions (promoters and/orenhancers). It can affect the rate of transcription and, consequently, the abundance of protein
– In introns. The mechanism of association is not clear but it could affect the use of an exon (splicing) or intron enhancers
– At the end of a gene (region 3' not translated). It could affect the stability of messenger RNA and, consequently, the abundance of the protein

2. Polymorphisms outside the gene (5' or 3'). It could affect the structure of chromatin, with implications on the expression of the gene and can even be suppressed.
3. Polymorphisms in or outside the gene. Specially in regions which are non-encoding or in what is mistakenly known as "junk" DNA that could affect the sequence and, in consequence, the function of some micro-RNA which regulates the expression of other genes.

Independent of the mechanism, an affected gene could not be directly implicated with a pathogenic mechanism, but it could be a gene that regulates the expression of genes that cause a certain pathogenic mechanism. While functional genomics are not thoroughly defined, a precise knowledge of this is impossible. Therefore, we will describe some of the genetic associations of RA and will discuss the possible effects of the described polymorphisms.
It is well known that the main genetic factor associated to the risk of developing RA, widely confirmed in different ethnic groups, is a polymorphism in the DRB1 locus,[50-55] which belongs to the class II region of the major histocompatibility complex (MHC), which encodes the beta chain of the HLA-DR molecule. This gene has more than 500 alleles; those that encode the Gln-Lys-Arg-Ala-Ala (QKRAA, 1 letter international code) amino acid sequence in the 70-74 residues of HLA-DRβ1 (shared epitope) are associated to RA. Of the autoimmune diseases, only type 1 Diabetes Mellitus is associated to a larger risk with polymorphisms in this locus (P=2.44×10^{-134}). Subsequently, other genes associated to RA have been found, some in all ethnic groups, while others vary depending on the population studied.
Up until recently, the genetic study of complex diseases such as RA was limited to 1 or a few genes for every research group. Sequencing of the human genome and the availability of genotyping, along with advanced statistical methodology, have made it possible to simultaneously study the patterns of genetic variation in all of the genome in populations of individuals.
Single nucleotide polymorphisms (SNP) are variations that occur once every 100-300 base pairs in the genomic DNA in the general population, both in encoding and non-encoding regions, and represent 90% of human genetic variations.[56-59] Some SNP have a great impact on biology and the response to environmental agents, including viruses, bacteria's, toxins, and drugs. Initial studies of isolated SNP identified polymorphisms associated to RA, which seem compatible with certain aspects of its pathogenesis.
Currently, genotyping can be done with large scale SNP microarray systems (LSSNP), which identify more than 500 000 SNP in all of the genome, encoding or non-encoding. LSSNP studies analyzed by the Haplotype Maping International Project (HapMap),[60] a public

database containing more than 2 million SNP with known and verified allele frequencies, are already contributing to the identification of the genetic variants that predispose to complex diseases such as RA,[61] some of which could vary according to the ethnic group. This, along with genetic expression studies done through genomics and/or proteinomics, will significantly contribute to define the pathogenesis of RA. Two recent studies with LSSNP, one in 2000 caucasian British patients with RA and 3000 healthy controls[61] and the other in Americans and Swedes,[62] confirmed that DRB1 is the main susceptibility locus for RA (P=3.44×10^{-76} and P<1×10^{-100}, respectively) and that the second susceptibility locus is the gene for the PTPN22 tyrosine phosphatase (P=4.9×10^{-26} and P=2×10^{-11}, respectively), which is also associated to other autoimmune diseases such as systemic lupus erythematosus (SLE) and type 1 diabetes mellitus.[61,63,64]

Other previously described polymorphims is RA were confirmed only in one or the other LSSNP studies (Table 1). In the British study there was an association with the genes that enconde for the alpha and beta chains of the interleukin (IL) 2 receptor (IL-2α, IL-2β; both, P=10^{-5}), and the genes for the TNF inhibitor protein (TN-FAIP2), granzime B (GZMB), proteinkinase C-θ (PKCθ), and a protease inhibitor KAZALD1; all with P=10^{-4}-10^{-5}. In the American/Swedish study there was no SNP found in these genes, but there was one in a 100 kb region of chromosome 9 where the TRAF1 genes and those encoding for complement fraction C5 (P=2.8×10^{-8}), apart from a positive, though lesser association in the genes of the CD40 receptor (P=3×10^{-6}), bradikinin 1 receptor (BDKR1, P=1×10^{-5}), a group of genes on chromosome 17 that encode chemokines CCL1, CCL3, and CCL8 (4×10^{-5}), and an SNP in a gene intron that encodes the STAT4 molecule (chromosome 2), of the cytokine signaling pathway. Any alteration in these genes could participate in the development of autoimmunity (Table 1).

A potentially interesting SNP, associated to RA in Asian patients is the peptidilarginine deaminase gene (PADI) 4.[65,66] This family of enzymes hidrolyzes imino groups from arginine to hidroxyl in proteins, transforming them to citrulline and produce the citrullinated proteins present in the organism (more below). Othe polymorphims associated to RA, also in Japanese patients, are the NFKBIL1, SLC22A4, and RUNX1 genes.[67]

From all of the above we can conclude the following:

– The main locus of association to RA (HLA-DRB) encodes one of the most important proteins in the triggering of adaptive immune responses
– The majority of additional polymorphisms associated to RA occur in genes that encode proteins that participate in the regulation of the immune response and/or inflammatory process
– Some RA associated polymorphisms occur in different genes that affect the antigenic structure of proteins (such as citrullination), tissue repair processes (such as KAZALD1), and others that possibly affect that response of the organism to immune auto-aggression
– In all of the described polymorphisms, only 2 (DRB1 and PTPN22) affect the protein sequence, and all of the others happen in non-encoding regions of DNA
– Some polymorphims associated to RA vary in different ethnic groups

TABLE 1. Principal Polymorphisms in Rheumatoid Arthritis[a]

Genes	Chromosome	SNP (S)	Sequence	Site	Functional Effect
DRB1	6p21	Many	Many	Exon	Peptide selection
PTPN22	1p13	rs2476601	1858C/T	Exon	R620W-gain
IL2RA	10p15-p14	rs2104286	A/G	Intron	(?)
IL2RB	22q13.1	rs743777	A/G	Intergenic	(?)
TRAF1-C5	9q33-q34	Several	Several	Intergenic/intron	(?)
PRKCQ	10p15	rs4750316	C/G	Intergenic	(?)
TNFIAP2	14q2	rs2771369	A/G	(?)	(?)
KAZALD1	10q24.1	rs10786619	C/T	Intergenic	(?)
CTLA-4	2q33	rs11571300	C/T	Intergenic	(?)
STAT4	2q	rs7574865	G/T	Intron	(?)
PADI4	2	NA	Several	Exons	¿Gain?

[a]See text for abbreviations.
SNP are referred to according to their NCBI and Affymetrix nomenclature.

Implications of the Genetic Findings in the Pathogenesis of RA. Functional Model

Everything indicates that RA is an autoimmune process that depends on CD4+ lymphocytes that induce chronic synovial inflammation; among other evidence, the response to cyclosporine A,[17,18] FK506,[19] and abatacept[39-44] supports this affirmation. These stimulate macrophages and fibroblasts to produce cytokines such as IL-1, IL-6, and TNF,[68] apart from proteases, leading to cartilage and subchondral bone degradation. However, that underlying causes that lead to the activation of these effector systems is unknown. Therefore, even with advances in genomics and proteinomics, the etiology of RA is still unknown and the knowledge of its pathogenesis is still superficial. The elaboration of a model that explains the pathogenesis of RA must take into account, in addition to the biologic findings, the function of the genes that present polymorphisms associated to RA and the mechanisms of action of some of the treatments with proven efficacy, but only the drugs with known mechanisms of action.

Role of the Shared Epitope

The shared epitope refers to the QKRAA (Gln-Lys-Arg-Ala-Ala) sequence of amino acids in residues 70-74 of the third polymorphic region of the HLA-DRβ1 chain. This chain is part of the HLA-DR dimer, one of the isotypes of human class II MHC. It is well known that the function of MHC II[69] is to present peptides that are derived from extracellular proteins to T CD4+ lymphocytes. The peptides presented on MHCII must bind to them and the 70-74 sequence of HLA-DRβ is found on the peptide-binding site. Different amino acids in the 70-74 sequence have, as a result, the capacity to bind different peptides; therefore, the main difference between individuals carrying the shared epitope and those who don't is the type of peptides presented by the HLA-DR molecule.

A detailed analysis was recently done on the sequence of the shared epitope,[52,53] and it was found that susceptibility depends strictly on the RAA (arginine-alanine-alanine) sequence on the 72-74 amino acids, while the K or R (lysine or arginine) residues in position 71 and, to a lesser degree, the Q residue (glutamine) in position 70, apart from contributing to the risk, is related to the production of autoantibodies and apparently to the severity of RA (Table 2). What is notable here is that the arginine (R) residue in this sequence has a positive charge, while alanine is neutral. In addition, lysine or arginine in position 71, both basic amino acids, increase the total positive charge in this region, while glutamine in position 70 is a polar, neutral amino acid. Therefore, the charge of the shared epitope is firmly positive, making it possible to predict that peptides that bind to this HLA-DR molecule must have a negative charge (that is, "acid" amino acids such as aspartic acid and glutamine) and not have any positively charged amino acids (lysine, arginine, and hystidine).

The abovementioned data is exemplified considering the sequences of amino acids in positions 70-74 in non-RA related alleles, which has aspartic acid (D) in the 70 position or glutamic acid (E) in the 71 position, both with a negative charge that counteracts the positive charge of arginine in position 72. Besides, in all of the unassociated alleles, the residues in position 74

TABLE 2. Risk of Rheumatoid Arthritis According to the DRβ1 Sequences[a]

Positions					Designation	Risk	Alleles DRβ1
70	71	72	73	74			
Q	K	R	A	A	S2P[b]	++++++	*0401
D	K	R	A	A	S2D[b]	+++++	*1303
Q	R	R	A	A	S3P	++++	*0101, *0102, *0404, *0405, *0408, *1001, *1402
D	R	R	A	A	S3D	+++	*1101, *1104, *12, *16
D	E/A	R	A	A	S1	++	*0103, *0402, *1102, *1103, *1301, *1302, *1323, *15
Q/R/D	R/K/E/A	R	A/G	R/E/Q/L	X	NS	*03, *0403, *0407, *0411, *07, *08, *0901, *1401, *1404

[a]A indicates alanine; D, aspartic acid; E, glutamic acid; K, lysine; L, leucine; Q, glutamine; R, arginine.
[b]Here they were subdivided (P and D are not used in the original description) to determine the presence of glutamine or aspartic acid in position 70 (S2P or D, respectively).
Susceptibility groups (S) were divided in 5 subgroups according to the amino acid sequence in residues 70-74 of DRβ1 corresponding to the risk of developing RA and having autoantibodies.
There are additional alleles that have not been classified for these sequences but that are much less frequent in the population.

(arginine/glutamic/glutamine/leucin, R/E/Q/L) have physicochemical characteristics that are very different from alanine. Therefore, the possible net result of all of this is that the alleles associated to RA would bind peptides with a less positive charge, with important implications on the targeting of immune responses, something that will be explained below.

B Lymphocytes, Citrullinated Proteins and the Antibodies Against Them

Of the antibodies characteristic to RA, the ones that appear earliest are those that recognize cyclic citrullinated peptides (CCP) and/or citrullinated fibrinogen.[70,71] Citrulline is a precursor in the biosynthesis of arginine, synthesized from ornitine with the addition of ammonia and CO_2. On the other hand, NO synthase hydrolyzes arginine to citrulline and NO.

Citrulline does not have codons or transfer RNA for its incorporation into proteins. Therefore, citrullinated proteins are the hydrolysis product of an imino group of the arginine residues on proteins by PADI, which has various isoforms and different patterns of tissue expression. As mentioned, the PADI4 gene has a SNP associated to RA in Asians.[66,72]

The imino group of arginine has a positive charge, which in citrulline is a neutrally charged hydroxyl. Therefore, hydrolysis of cytruline to arginine neutralizes its positive charge in proteins. As a consequence, citrullinated peptides have a less positive charge than the same sequence with arginine, something that necessarily affects their binding to the MHCII molecule (such as HLA-DR). As has already been mentioned, the 70-74 sequence of DRβ1 is found in a site which is crucial for the binding of peptides to HLA-DR, which in individuals carrying the shared epitope allele has a positive charge, favoring the binding to peptides with a negative or neutral charge (such as citrullinated peptides) which then would be presented to autoreactive T lymphocytes. Some studies have confirmed that in patients with RA who carry the shared epitope there is an increase in the response of T lymphocytes to citrullinated peptides.[52,65,71]

Though the functional consequences of the PADI4 polymorphism is unknown, this could positively or negatively affect the capacity of protein citrullination and possibly the immunogenicity of some proteins; on one hand, for its presentation to T lymphocytes by the HLA-DR molecules with a shared epitope and, on the other, for the anti-CCP antibody specificity. Concretely, specific B lymphocytes against citrullinated peptides would capture them through their receptor (surface immunoglobulin or BCR). This would be followed by their endocytosis and partial degradation (processing) to peptides (also citrullinated), some of which could bind to the HLA-DR and be presented to autoreactive T lymphocytes, something that is necessary, though clearly insufficient for the initiation of autoimmunity. Most of the individuals who carry the shared epitope do not have RA, unless they also carry other susceptibility genes in a still unpredictable combination mosaic.

Genes that Encode Activation Cell Regulation Proteins. Heroes and Villains

It is important to be careful in the interpretation of the genetic data because they must always be confirmed and their meaning is difficult to determine. Nonetheless, some of the associations seem genuine and the functional information in them indicates potential pathogenic implications of potential importance.

Of the susceptibility genes for RA, many encode proteins that participate in the activation or inhibition of cell functions, specifically those on T lymphocytes. The second susceptibility gene for RA, tyrosine phosphatase PTPN22, negatively regulates the activation of T lymphocytes. The 2 most frequent alleles of PTPN22 are PTPN22-1858C and 1858T (with a single substitution on the 1858 nucleotide, which results in a difference of 1 amino acid in 620 position; arginine for 1858C (PTPN22-620R), and tryptophan for 1858T (PTPN22-620W). The main proteins dephosphorylated by PTPN22 in T lymphocytes are tyrosine kynases (PTK) Lck and ZAP70.[73] Both PTK have 2 main phosphorylation sites, one that inhibits its enzymatic function and the other one that activates it. Therefore, Lck 505P is inactive, while Lck 394P is active. Also, ZAP70-319P is inactive and ZAP70-493P is active. PTPN22 dephosphorylates the active forms: Lck 505P and ZAP70 493P, making its effect an inhibition of the function of these PTK and, in consequence, of the activation of T lymphocytes through the TCR (Figures 1 and 2).

Unexpectedly, a recent study found that the RA associated PTPN22-620W allele has a larger catalytic activity than 620R[74]; that is, it inhibits in a more efficient manner the signaling of the TCR. Therefore, its activation threshold in persons who are carriers of PTPN22-620W is higher, for which patients with autoimmune diseases would have, paradoxically, an immune response of lesser intensity. Therefore, the possible role of this single amino acid change in PTPN22 could be explained in 2 alternate, though not exclusive ways. On one hand, the T lymphocytes of patients with RA would have a reduced capacity to respond to antigens; however, during ontogeny this would affect the efficiency to eliminate autoreactive clones. Therefore, though the lymphocyte activation threshold in RA would be high, the number of autoreactive clones would be elevated (Figure 2). Alternatively in the periphery, in carriers of the susceptibility allele, the

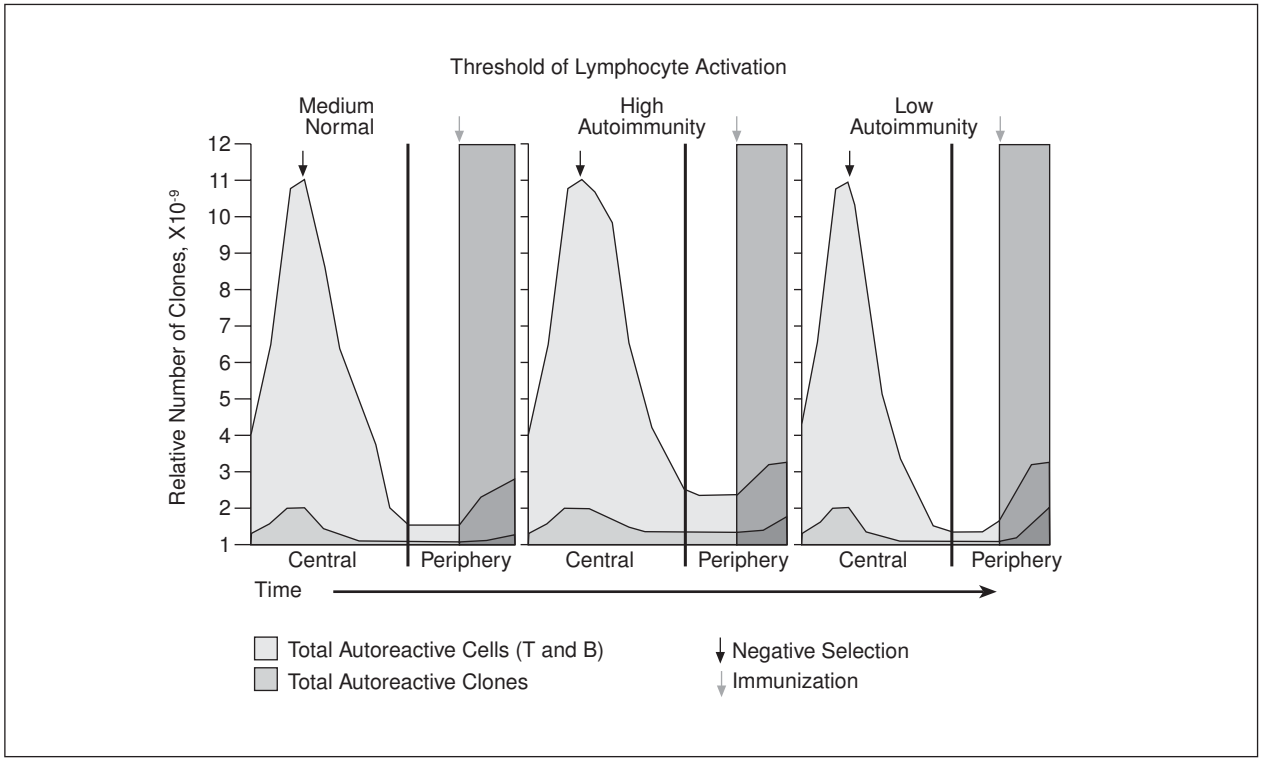

Figure 2. *Negative selection of B and T autorreactive lymphocytes according to their activation threshold. What happens in healthy subjects can be seen on the left. In the center, an excess of autoreactive clones due to a high activation threshold that leads to the persistence of autoreactive clones that are overrepresented in susceptible individuals who carry PTPN22 620W. On the right, a hypothetical situation in individuals with a low activation threshold, with low numbers of autoreactive clones but an increased capacity for activation.*

induction of regulating T lymphocytes would be less efficient. The first possibility is compatible with a model of spontaneous arthritis, similar to RA in mice, in which a mutation in ZAP70, which diminishes its capacity for signaling (similar to what happens in individuals with PTPN 620W), reduces clonal deletion in the thymus, allowing autoreactive T lymphocytes to pass into the peripheral blood and eventually cause autoimmune arthritis.[75]

Other polymorphisms associated to RA are the ones present in the genes that encode the alpha and beta chains if IL-2R, in both cases, in an intron.[61] This receptor receives signals from one of the main T lymphocyte cytokines (IL-2). At the start of the immune response, IL-2 is indispensable for T and B lymphocyte proliferation; however, once activated, IL-2 induces death by activation, making it necessary for an immune response with a self-limited course. Besides, IL-2 is essential for the differentiation of a subgroup of regulating T cells (Treg), which modulate the immune response.[76] The congenital absence of IL2Rβ and/or IL-2Rα in experimental models leads to the development of autoimmune diseases.[77]

Other polymorphisms associated to RA occur in genes that encode inhibitors of the inflammatory process, such as TNFAIP2 and NFKBIL1, in introns.[78,79] While another polymorphism is closer to the gene that encodes for PKCθ,[61] the main kynase of serin and threonine that transduces positive signals from the T cell receptor (TCR). The polymorphims in the TRAF1-C5 are important because TRAF1 participates in the signaling of TNF and the absence of C5 in mice makes them resistant to the experimental development of arthritis. Finally, other polymorphisms with an unknown meaning are found in the SLC22A4 and RUNX1 genes,[67] which are also present in other autoimmune diseases.

The combined loss of some of these genes and the gain of others, with the shared epitope, could explain the pathogenesis of autoimmunity in RA. In addition, different combinations of these polymorphisms could lead to subgroups of RA that could vary in their clinical behavior and/or in their response to different types of therapy.

In conclusion, for the pathogenesis of RA, the fact that the main susceptibility gene (DRB1) is an MHCII molecule has important implications. The function of MHCII is to present peptides to T $CD4^+$ lymphocytes, the initiators of the adaptive immune response[69] (see below). If the T lymphocytes in RA patients have a high signaling

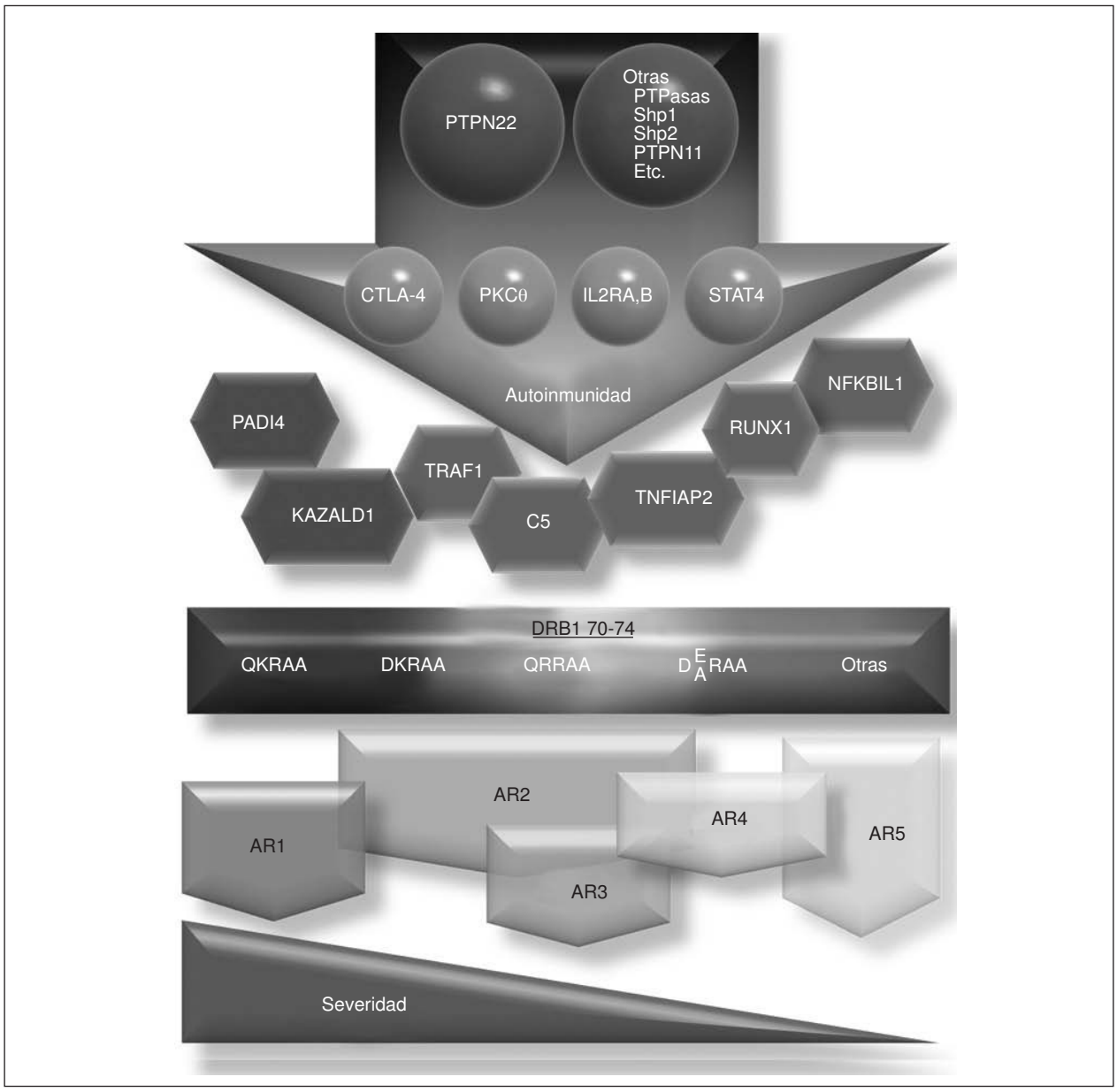

Figure 3. *Pathways to autoimmunity and the clinical and pathogenic mosaic of rheumatoid arthritis. See text for explanation.*

threshold through their TCR due to an effect of PTPN22 (dephosphorylation) or due to defective signaling (PKCθ), the result would be a failed clonal deletion, with an increase in the autoreactive clones. If, on the other hand, there is an increase in citrullinated proteins (PADI4 polymorphism), up taken by B lymphocytes and presented by DRB1 with the QKRAA sequence, which more easily present the citrullinated peptides, autoreactive T and B lymphocytes would be activated in a reciprocal manner. The latter are the ones responsible for the secretion of anti-CCP antibodies, while the former, for some still obscured reason (see below for a possible explanation), would migrate to synovial tissue.

Defects in Treg lymphocytes, due to errors in signaling of the TCR and/or IL-2R, with defects in the control of the inflammation, due to an increased signaling of TNF and other inflammatory cytokines (TNFAIP2 and NFKBIL1), would make a mild antigenic stimulus result in a magnified inflammatory response, which in individuals with defects in tissue repair (KAZALD1) would favor the early development of erosions (aggressive course RA). All of this hypothetical sequence of events is correlated

with different findings in patients with RA, some of which don't necessarily have a direct genetic translation, but are very probably indirect effects of the defect in the abovementioned genes. The predominant T lymphocytes in the synovial infiltrate of RA are $CD4^+$ and the main cytokine they secrete is IL-17,[68] which induces proinflammatory cytokines, such as TNF, IL-1, and IL-6, and chemokines, such as IL-8, which attract PMN. The activated macrophages in the synovium or RA cause tissue damage through TNF and IL-1, which induces the secretion of collagenase and elastase by synoviocytes, and leads to the degradation of cartilage and bone. Persistent inflammation with TNF induces neovascularization, synovial hyperplasia, and fibrosis (pannus); also, together with RANK, activates osteoclasts, which leads to bone resorption. The duration of the inflammatory process is proportional to the destruction of cartilage, bone, tendons, and ligaments. IL-6 induces acute phase proteins, which are: pentraxins (C-reactive protein [PCR] and amyloid P [SAP]), complement, fibrinogen, apart from antibodies, and autoantibodies (such as rheumatoid factor and anti-CCP antibodies) by B lymphocytes and plasma cells, promoting the damage mediated by immune complexes. In fact, IL-6 is the direct or indirect cause of many of the systemic manifestations of RA.

A still unclear aspect, which is a necessary approach to explain the therapeutic efficacy of rituximab, is the role that B lymphocytes play in the pathologic process of RA. It has been proposed that this could be due to its function as an antigen-presenting cell (APC) to T lymphocytes in the synovial membrane. Because in the synovium (and in other immunological processes) there are other APCs, such as dendritic cells, with a better capacity for T cell activation, the role of B lymphocytes in RA would only make sense if we consider it as an APC that particularly presented citrullinated peptides and that the response against them, both in T as in B lymphocytes, plays a predominant role in RA (though not in all of the patients, because only around 25% respond to rituximab).

A possible mechanism of action of rituximab in RA could be the elimination of anti-CCP antibody-producing B lymphocytes, if their role in the pathogenesis of RA is similar to that of a murine arthritis model, in which an autoantibody against a ubiquitous protein[80-82] passively transfers the disease to healthy mice. In spite of beginning with autoantibodies, arthritis is established when, secondarily, the synovial membrane is infiltrated by T lymphocytes, macrophages, and other APCs. This process also depends on the participation of the C5 fraction of complement, Fcγ receptors, TNF, and mast cells. Even though all of these elements have been identified as essential for the development of disease, the precise role of each one of them is still unclear.

Clinical Utility of Knowing the Molecular Alterations in RA

Many of the mechanisms of structural damage in RA are already known and some of its biologic mediators have been completely identified. It is clear that the functional prognosis of RA depends in a direct way of the biologic control of inflammation, which has to be done in the least amount of time possible, because it depends on 2 main variables: *a)* an early diagnosis, and *b)* choosing the best therapeutic option for each patient. Currently, none of them is within easy reach.

An early diagnosis of RA continues to be a problem; first, because there are no paraclinical tools to establish or discard this diagnosis, which is still based in the clinical manifestations according to the criteria proposed by the American College of Rheumatology (ACR), whose main problem is that an essential aspect of almost every point is chronicity (more than 6 weeks), apart from the fact that they only apply to patients with active disease because they require the direct observation of inflammation by the physician.

A good clinician (any experienced rheumatologist) can predict, with a good degree of certainty, that a patient has RA even before he fulfills the ACR criteria. Though this makes establishing a more or less early treatment easier, it doesn't solve the problem of not being able to predict which is the best treatment for each patient individually. Because of this, several groups have dedicated themselves to defining what is now called early RA, which is useful, but not for epidemiological studies, because only patients complying with the ACR criteria can be considered as valid; what leaves those patients with a good response out of the picture is the fact that they did not fulfill the necessary number of criteria to be considered as RA (in spite of being authentic cases). Patient selection based on the positivity to anti-CCP antibodies is a substantial step forward with respect to other paraclinical tests. Nonetheless, even though anti-CCP can be found in early RA, not all RA patients have them and there are false positives to be found.

The chronicity component of the criteria is another aspect that affects clinical and therapeutic studies of RA, because at the moment of inclusion into a study and the formal start of treatment, a patient with RA has spent more than 6 weeks with synovitis, a time in which substantial structural damage can occur, because RA seems to be in a subclinical form for weeks or months before it is detectable through physical examination.

Apart from contributing to the knowledge of its pathogenesis, the genetic and molecular profiles of RA can be used as prognostic and susceptibility markers as well as indicators of response to different therapies or to design new drugs. Isolated genetic association studies are useful, but the number of genes that one can study is limited. A solution to this is SNP microarray genotyping

in order to identify genetic variants associated to RA, which can differ among ethnic groups. We have already referred to 2 recent massive genotyping studies using this methodology in patients with RA.

Another tool to know the molecular profile of a disease is proteomics, which instead of studying genes analyzes its final product: proteins, which reflect the functional status of an organism. In the cell, genes are transcribed to messenger RNA (mRNA) which encodes the sequence of 1 or more proteins per gene, depending on the selection of exons in the mRNA sequence through a cut and paste mechanism, selectively eliminating some of the exons (alternative splicing), which when translated to proteins lead to isoforms. Many proteins suffer, in addition, posttranslational modifications (PTM), or form olygomers with other proteins. Therefore, depending on their PTM, a gene can generate different proteins with different functions.

A proteoma is the total protein complex expressed by a genome, which identifies the degree of expression, isoforms, PTM, interactions, and protein localization, with which it contributes to the knowledge of complex diseases. A proteoma is dynamic and varies with the environmental conditions of the cell, tissue, and organism. The comparison of proteomic patterns in the serum and synovial fluid of patients with RA will allow for the design of new diagnostic and treatment follow-up tools, leading eventually to specific therapies.

An NCBI database search with the words "arthritis" and "proteomics" showed 54 publications up to August 2007, while a more restrictive search with the words "rheumatoid," "arthritis," and "proteomics" showed 36 publications. In comparison, when the words "cancer" and "proteomics" were used, 1990 publications were found. Proteomic studies in serum and synovial fluid of different rheumatic diseases identified acute phase proteins.[83] For example, in RA the levels of CRP indicate the degree of inflammation, but are not a reflection of the severity of the disease nor are they sensitive enough to measure response to treatment.[84-86] SAP is present in serum, plasma, and synovial fluid of patients with RA, but not in osteoarthritis (OA).[87] Some isoforms of fibrinogen and calgranulins A, B, and C are associated to RA[88] and the latter are also found in spondyloarthritis, but none on OA.[83]

Liau et al identified 33 potential marker proteins in the synovial fluid of patients with RA,[89,90] including members of the S100 protein family, and other proteins such as osteopontin,[91] cyclophilin (the target of cyclosporine A), cathepsin B among others that have been found elevated in the synovial fluid of patients with RA and erosions. Proteomics also allows for the identification of the autoantibody pattern, which in healthy individuals could predict autoimmune disease. In addition, the autoantibody profile in RA could also predict the course of a disease that has already been diagnosed.[92]

In summary, RA is a disease that mainly affects productive individuals. Its prevalence is 0.1% to 0.5% predicting that worldwide there are more than 100 million persons affected by this devastating disease, with 10% of patients following an unremitting course that is resistant to conventional treatment, representing enormous costs in every sense. Therefore it is fundamental to optimize the diagnostic, prognostic, and therapeutic response means, which implicates without a doubt, the knowledge of its pathogenic mechanisms.

The knowledge of the genetic variants of RA, applied individually, will allow the prediction of which patients will respond and which will be resistant to different therapeutic agents, allowing for a better assignation to treatment, with less therapeutic failures (50% less), a shorter time of response, a greater chance of remission, and, in consequence, less structural damage. The systematic use of genetic and/or proteomic patterns will also be useful for the design of new treatments. Changes in the proteomic patterns could facilitate the follow-up of the biologic behavior of RA according to modifications in disease activity.

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