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Future perspective for diagnosis in autoimmune diseases

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ABSTRACT

Human beings have taken successive approaches for the understanding and management of diseases. Initially brewed supernatural concepts and mystical procedures, a vigorous scientific approach has emerged on the grounds of fundamental disciplines such as anatomy, microbiology, biochemistry, physiology, immunology, pathology, and pharmacology. The resulting integrated knowledge contributed to the current classification of diseases and the way Medicine is carried out today. Despite considerable progress, this approach is rather insufficient when it comes to systemic inflammatory conditions, such as systemic lupus erythematosus, that covers clinical conditions ranging from mild pauci-symptomatic diseases to rapidly fatal conditions. The treatment for such conditions is often insufficient and novel approaches are needed for further progress in these areas of Medicine. A recent breakthrough has been achieved with respect to chronic auto-inflammatory syndromes, in which molecular dissection of underlying gene defects has provided directions for target-oriented therapy. Such approach may be amenable to application in systemic auto-immune diseases with the comprehension that such conditions may be the consequence of interaction of specific environmental stimuli and an array of several and interconnected gene polymorphisms. On the bulk of this transformation, the application of principles of pharmacogenetics may lead the way towards a progressively stronger personalized Medicine.

Key words: autoimmunity, pharmacogenetics, gene polymorphism, auto-inflammatory diseases, systemic lupus erythematosus, rheumatoid arthritis.

HISTORICAL PERSPECTIVE IN DIAGNOSIS PARADIGM

Since ancient times, human beings have struggled to provide help to those suffering from the diverse illnesses that affect mankind and other living beings. Primitive paradigms were mainly based on instinct, mysticism and supernatural approaches. Dance rituals and magic medicines were the answer that those early professionals provided to the sufferers. With the advent of the early founders of the contemporary medical science in the Mediterranean area, a rational, observational and ethical approach was progressively established. Accordingly, the diagnostic and therapeutic approaches were

then modified towards more effective procedures, including recommendations related to life style and hygiene. However, still at those early times the primitive theories for health and disease were largely based on beliefs, such as the miasmas and circulating humors (blood, phlegm, black and yellow bile). Therapeutic interventions included baths at special temperatures, boiling oil, ointments, bloodletting, suction cups and so on (Lyons and Petruccelli 1978).

With the advent of Renaissance and the rise of the contemporary scientific system, as we know it today, different fields in natural sciences started to contribute



and other scientific fields contributed to the building of a new body of knowledge that progressively allowed the conception of novel medical paradigms that gradually would replace the old ones. Miasmas and circulating humors were not appropriate to the facts brought up by objective investigations in microbiology, anatomy, physiology, and the nascent clinical chemistry. The new concepts would re-shape the established nosology (disease classification system). The art of clinical examination would also change with the aim of providing clues that would guide physicians in classifying their patients according to the new nosologic classification system. Academic clinical practice would feed back this emerging nosologic classification system and vice-versa. In due time, patients were diagnosed in a classification system that included conditions such as bacterial pneumonia, left heart failure, acute hepatitis, diabetes mellitus, gouty arthritis, and so on.

It is common practice that the application of novel paradigms into practical activities lags behind the scientific advance pace. In fact, for a considerable period of time, many of the involved professionals are bound to reject the novel ideas or to under-estimate the magnitude of the coming changes. To illustrate this point, let us suppose that a regular physician from the 17th century was to be confronted with a medical assessment of a fiction patient as of today (Table I). We can easily expect that this renaissance mind would be confused with mixed feelings of wonder and suspicion.

NOSOLOGIC CLASSIFICATION SYSTEM AS A FUNCTION OF SCIENTIFIC AND TECHNOLOGICAL PROGRESS

Scientific paradigms, systematic classifications of phenomena, and technology operate in an interactive mode in which each one of these three elements influences the progress of the other two (Fig. 1). Advances occurring in any of these three elements are expected to influence the two other elements. New methodological tools allow a closer look at the natural phenomena, which may contribute to improve the classification of the system and eventually confirm or challenge the established scientific paradigm. Novel scientific paradigms push the develop-

paradigms along the last four centuries. The same is true for Biology and related areas, including Medicine.

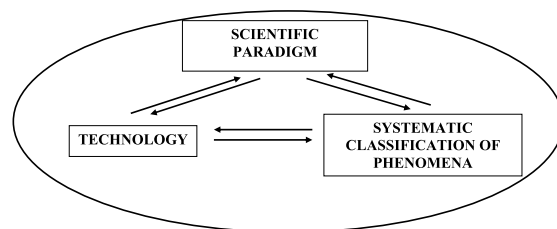


Fig. 1 – Mutual and virtuous interaction among scientific paradigms, technical advances, and classification systems.

CRITICAL APPRAISAL OF THE CURRENT NOSOLOGY OF SYSTEMIC RHEUMATIC DISEASES

The current nosologic classification of systemic rheumatic diseases has emerged in the middle of the 20th century. During the first decades of the last century, the strong influence of Paul Erlich has pushed the scientific community towards the concept of “horror auto-toxicus”, which was erroneously understood as the impossibility of the immune system to recognize self-constituents. Systematic experimentation progressively disclosed, however, consistent evidence that, under certain circumstances, the immune system may set up a vigorous response against self tissues and organs. Some of these seminal experiments included the immunization of rabbits with thyroid tissue that elicited auto-reactive T lymphocytes and the destruction of the thyroid gland by an intense inflammatory infiltrate. In addition, the disease could be transferred to healthy animals by inoculation of lymphocytes from the immunized rabbits (Rose 1988, Doniach and Roitt 1988).

As a hint on how intense was the dispute in this field, one can evoke the episode in which the American hematologist William Harrington self injected plasma from a patient with putative immunologic thrombocytopenic purpura. After a few hours, the platelet count started falling down reaching a nadir of 7,000/mm³ coincident with an episode of generalized tonic-clonic convulsion. Fortunately, the devoted researcher recovered with no major sequel (Harrington et al. 1951).



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TABLE I
Fiction patient presented to a physician at the middle age era.

Systemic blood pressure: 150 × 95 mmHg
Blood hemoglobin: 9.5 g/dL
Blood lymphocyte count: 340/mm ³
Serum gamma-glutamyl transferase: 24 U/L
Blood glucose: 93 mg/dL
Estimated systolic pressure in pulmonary artery: 75 mmHg
Capillary ectasia and deletion at the nailfold
Antinuclear antibody 1:640 with coarse speckled nuclear pattern
Anti-RNP antibodies: 1/10,240
High-resolution thorax tomography → ground-glass images in lung bases

and the LE cell phenomenon (Hargraves et al. 1948). These two pioneer autoantibodies set the road for a vigorous research field that would flourish for the whole 20th century (Tan 1997, Strassburg and Manns 2002, Sherer et al. 2004). The steady progress in the demonstration of a series of clinically relevant autoantibodies helped pave the way for the nosologic classification system for the new family of diseases, the so-called autoimmune diseases (Chan and Andrade 1992, Tan and Chan 1993, Dellavance et al. 2005).

Nowadays, the concept of autoimmunity is well established (Schwartz 1993). Diseases with widespread involvement are classified as non-organ specific autoimmune diseases. Examples of these include systemic lupus erythematosus (SLE), systemic sclerosis, dermatomyositis, polymyositis, rheumatoid arthritis, and Sjögren's syndrome. Those with a more restricted organ involvement are designated organ-specific autoimmune diseases. Examples of these are autoimmune thyroiditis, pemphigus vulgaris, myasthenia gravis, autoimmune hepatitis, and chronic biliary cirrhosis. Although this classification system has provided a reasonable framework to differentiate and manage these patients, it is not without flaws. In fact the extent to which a given disease is restricted to a certain organ is quite variable and many organ-specific autoimmune conditions present various degrees of systemic involvement. We can take autoimmune hepatitis type I as an example, in which skin and joint involvement is quite frequent (Manns and Vo-

syndrome, in which many patients show evidence of restricted involvement of salivary and lachrymal excretory glands (Fox 2005, Barcellos et al. 2007). Rheumatoid arthritis and polymyositis are also frequently restricted to sinovial joints and skeletal muscles, respectively (Bouysset et al. 2005, Briani et al. 2006).

Another problematic point in the current classification system is that many patients present features that overlap two or more of the defined autoimmune diseases. It is not unusual, for example, that a patient with systemic sclerosis presents isolated features suggestive of systemic lupus erythematosus, such as swollen fingers, Raynaud's phenomenon, and esophageal dysmotility (Furtado et al. 2006). In some cases, there is concomitant full-blown presentation of two or more of these conditions. The current classification system accommodates those cases as overlap syndromes (Rodriguez-Reyna and Alarcon-Solano 2006). In addition, the association of an organ-specific and a non-organ specific disease is quite frequent. For example, patients with rheumatoid arthritis are frequently affected by thyroid autoimmune diseases (Sommerfeld 2006).

However, the most crucial deficiency in the current autoimmune disease classification system is the failure to appropriately addressing the large heterogeneity within each nosologic entity. The diagnosis of SLE *per se*, for example, is not enough to provide an accurate prognosis of that particular patient or of which type of treatment should be started. There is a wide



suffer no major impact on life style and no major interference with life perspectives. Some other patients with SLE may present serositis, thrombocytopenia, deforming skin involvement, and incapacitating fatigue. These may experience considerable interference with life style and professional and social performance. Finally, some other patients equally diagnosed as SLE may present severe and life-threatening manifestations, such as CNS vasculitis, alveolar hemorrhage or severe class IV glomerulonephritis. These few examples show clearly that it is not enough to say that a given patient has SLE. An accurate nosologic classification system should be able to narrow down the spectrum of manifestations expected for that diagnostic label and, therefore, allow for an accurate prediction of the disease course and the correspondent therapeutic planning for the patient (Fig. 2). Part of the complexity of disease phenotype is determined by non-genetic elements, such as environmental exposure, life style, quality of the medical care, compliance to the treatment, social and cultural level and existing co-morbidity. However, lessons from animal models and from clinical research have pointed that gene polymorphism also plays an important role in the determination of specific autoimmune phenotypes.

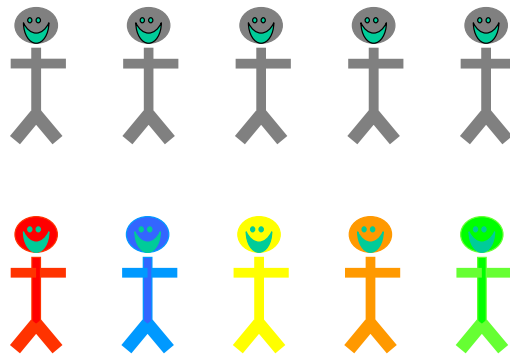


Fig. 2 – Unraveling well-defined sub-phenotypes underneath broad-label general syndromes. Different patients with the label of a general syndrome (upper panel), like SLE for example, present specific clinical presentations with peculiar prognostic and therapeutic implications (lower panel).

toimmune diseases, such as rheumatoid arthritis, systemic sclerosis, polymyositis, and autoimmune liver diseases?

It seems that our contemporary body of knowledge and available technology are clearly not suitable for such a reformulation of the current autoimmune diseases classification system. However, one can always take the neighbor’s experience as a perspective for one’s own development. In fact, the case of the familial periodic fever syndromes, the so-called auto-inflammatory syndromes, may provide a clue to the diagnosis dissection needed for the field of autoimmune diseases.

THE EXAMPLE OF THE AUTO-INFLAMMATORY SYNDROMES

The recent development in the field of the hereditary auto-inflammatory syndromes is quite exciting. For many decades, the Mediterranean familial fever was the only form of hereditary periodic fever syndrome to be clinically and epidemiologically individualized. Over the past 10 years or so, other members of this family of conditions, now designated as auto-inflammatory syndromes, have been defined at the molecular level.

The hereditary periodic fever syndromes are characterized and defined by the following features: recurrent and self-limited episodes of fever and systemic inflammation with no apparent cause; variable involvement of skin, joints, serous membranes and internal organs; the presence of hereditary component; and the development of amyloidosis as a late complication (Grateau 2004). The prototypic and most common auto-inflammatory syndrome is Familial Mediterranean Fever. As suggested by the name, it affects predominantly subjects with descent from the Mediterranean area. It is an autosomal recessive condition caused by mutations in the *MEFV* gene in region 16p13.3. Heterozygous subjects may present mild traits of the disease. The *MEFV* gene codes for marenostatin, a protein that is able to modulate the effect of caspase 1 on the transcription regulation of IL-1 β gene. Mutations in *MEFV* gene may cause a deficient inhibition of IL-1 β expression and therefore induce a pro-inflammatory status (Zaks et al. 2003. Schaner and

Should the SLE entity be split into several other



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TABLE II
Molecular definition of auto-inflammatory syndromes.

<ul style="list-style-type: none">• Familial Mediterranean Fever<ul style="list-style-type: none">– 26 mutations in <i>MEFV</i> gene – 16p13.3 (most in exon 10, nt 680 & 694)– Dysfunction of marenostirin (also known as pirin)– Impaired inhibition of IL-1β• Cold Induced Auto-Inflammatory Syndromes – 1 (CIAS-1)<ul style="list-style-type: none">– Mutations in <i>CIAS-1</i> gene– Dysfunction of criopirin \rightarrow increase in IL-1β– Muckle-Wells syndrome (MWS)– Familial cold urticaria (FCU)– Chronic Infantile Neurologic, Cutaneous and Articular Syndrome (CINCA) or Neonatal Onset Multisystemic Autoinflammatory Disease (NOMID)• Traps – Tnf Receptor Associated Periodic Syndrome<ul style="list-style-type: none">– Mutations in <i>TNFRSF1A</i> gene (12p13) \rightarrow disturb the first two cystein-rich domains of the 55-kDa TNF receptor– The defective 55-kDa soluble TNF receptor is incapable of neutralizing TNF• Hyper-Igd Syndrome<ul style="list-style-type: none">– Mutations in <i>MVK</i> gene (12q24) \rightarrow deficiency in mevalonate Kinase \rightarrow increase in IL-1β synthesis

requirements for classification as auto-inflammatory syndromes and their underlying molecular defects have been identified. These include the Cold Induced Auto-inflammatory Syndromes – 1 (CIAS-1) (Aróstegui et al. 2004), the TNF Receptor Associated Periodic Syndrome (TRAPS) (Masson et al. 2004, Aganna et al. 2003), and the Hyper-IgD syndrome (Prietsch et al. 2003). A preliminary list of molecular defects and mechanisms of the auto-inflammatory syndromes is depicted in Table II.

Would it be possible to adapt the molecular model of the auto-inflammatory syndromes to the classification of autoimmune diseases? The case of Autoimmune Lymphoproliferative Syndrome (ALPS) is quite compelling. This condition is characterized by adenomegaly, splenomegaly, autoimmune cytopenias, autoantibodies, and several SLE-like traits (reviewed in Worth et al. 2006). It is caused by mutations in genes involved in the Fas-mediated apoptosis pathway. Subtypes of ALPS with subtle clinical differences have been identified according to the specific molecular defect. Type Ia is caused by mutations in the *TNFRSF6* gene (Fas – CD-

type 2b is caused by mutations in the *CASP8* gene (caspase 8) (Worth et al. 2006). Although ALPS may have a molecular definition for some cases labeled as ALPS, it is clear that the vast majority of SLE patients do not have a defect in the Fas-mediated apoptosis pathway. However, other molecular mechanisms are long known to be associated with SLE, as is the case for congenital deficiency of early components of the complement system (C1q, C2, and C4) (Worth et al. 2006). Still, these examples represent a negligible fraction of SLE patients. Nonetheless, these examples are signaling that it may be possible to segregate specific clinical phenotypes within a very heterogeneous syndrome like SLE and to associate them with novel relatively homogeneous clinical phenotypes and specific molecular defects. It has become progressively evident that the genetic regulation of autoimmune diseases is extremely complex, and that the establishment of an autoimmune status is conditioned by the interaction of several dozens of alleles. For the last 15 years, an increasing number of gene polymorphisms have been associated with susceptibility or specific clinical features in several autoimmune diseases. In the case of



binding lectin, angiotensin converting enzyme, DNase I, among others (Alarcon-Riquelme 2005).

It is relevant for the present issue that the importance of gene polymorphism is not restricted to the determination of disease susceptibility, but it has also been associated with disease phenotype in human and murine SLE (Nolsoe et al. 2005, Qi et al. 2005, Johansson et al. 2005). In that sense, gene polymorphism may indeed contribute to the dissection of a general syndrome like SLE into sub-phenotypes with distinct clinical, prognostic and therapeutic features. However, it is increasingly apparent that several gene polymorphisms must concert interactively to modulate each specific phenotype. Notwithstanding this complexity, it is possible that the most relevant genes involved in the determination of a given autoimmune phenotype would be eventually amenable to simultaneous analysis in some sort of multiplex platform. In fact, the challenge of a simultaneous and integrated analysis of multiple metabolic pathways is hard to be achieved with the standard research methodology. In this respect, the novel and powerful tools provided by large-scale methodological platforms may prove to be helpful.

THE POTENTIAL CONTRIBUTION OF GENOMICS, TRANSCRIPTOMICS AND PROTEOMICS

As opposed to the traditional approaches in biochemistry, microbiology, and immunology, the last two decades have brought large-scale methodologies that allow the simultaneous analysis of thousands of parameters in a single and even in multiple parallel biological specimens. As opposed to the traditional approach of addressing a single or a few analytes, these methodological platforms offer a comprehensive evaluation of hundreds or thousands of parameters that are collectively expressed as specific patterns associated with certain biological conditions. These complex patterns are sometimes referred to as biological signatures. The science of pattern recognition is long known and deals with the ability of identifying an object or situation by the comprehensive appreciation of the whole instead of the details (Bolinger and Ahlers 1975). This kind of ability is responsi-

be applied to the definition of healthy and diseased biological states. These large-scale methodologies have paved the way for the development of genomics, transcriptomics, and proteomics according to the kind of analyzed target, genomic DNA, messenger RNA, and proteins, respectively.

Although the term is relatively new, proteomics has been around for several decades. Classical Tiselius electrophoresis and polyacrylamide gel electrophoresis (PAGE), under reducing and non-reducing conditions, were the first means of discriminating proteins in a complex sample. The use of 2-dimensional electrophoresis increased the discrimination power to an order of hundreds or thousands. The use of antibodies as probes in western blotting and Protein A-Sepharose facilitated immunoprecipitation brought considerable specificity to this approach. More recently, the use of mass spectrometry coupled to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) has greatly increased the power of proteomics analysis (Buetow et al. 2001). As an example, the proteomic analysis of plasma may provide specific profiles for certain kinds of clinical conditions, such as early breast and prostate cancer (Ornstein et al. 2004, Martin 2006). A similar approach has been applied to the definition of proteomic signature of the inflammatory status in autoimmune conditions, such as SLE and Wegener's granulomatosis (Bennett et al. 2003, Stone et al. 2005).

Genomics has allowed for the sequencing of the genomes of hundreds of species, including the human species. Detailed maps have been elaborated for known genes, expressed sequence tags (ESTs), variable number tandem repeats (VNTR), and single nucleotide polymorphisms (SNPs). These maps are fundamental tools for linkage of the genetic information to disease susceptibility, disease phenotype, and pharmacogenetics (Belmont and Gibbs 2004). In parallel, the planar array technology has provided the possibility of probing one or more biological samples for hybridization with thousands of available sequences of the genome, thereby allowing the establishment of specific patterns for biological samples in terms of VNTR, SNPs, and other forms of gene poly-



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assay. The impact of this methodology may be readily recognized by verifying the enormous number of publications on gene polymorphism of a great variety of genes in the last 10 years, as compared with the primary focus on polymorphism of the HLA system in the past decades.

The reverse transcription of sample messenger RNA into complementary DNA (cDNA) has made it possible to analyze the transcriptome, i.e., the gene expression pattern of a given biological sample. It is well appreciated that the full repertoire of genes of a given species is not used in each cell of the organism. In fact, only a certain assembly of genes is expressed in a given cell type and at a given physiologic status. The gene expression pattern of a given cell contributes to its specific phenotype. For instance, erythroid precursors synthesize hemoglobin and neurons synthesize neurotransmitters. As cells move from a physiologic to a pathologic status, the gene expression pattern and the protein expression profile change accordingly. The cDNA microarray technology has taken advantage of the gene expression pattern, to help distinguishing normal from tumoral tissue in lung, breast, stomach and other forms of cancer (Martin 2006, Brennan et al. 2005). Gene expression and protein profile are expected to be also useful in monitoring the therapeutic gene modulation in several conditions (Wulfschlegel et al. 2004). In SLE, microarray analysis of peripheral blood mononuclear cells has allowed the definition of a characteristic expression signature associated with genes regulated by Type I IFN, and this pattern is associated with disease activity (Belmont and Gibbs 2004). Proteomic analysis of biological samples has been also applied to the detection of several types of cancer (Ornstein et al. 2004, Liotta and Petricoin 2006) and to the assessment of the activity status of certain inflammatory diseases (Stone et al. 2005).

These novel tools have readily contributed to the understanding and managing of diseases associated with cancer and simple genetic syndromes. Applications of this methodology are emerging also for the field of autoimmune diseases. The determination of polymorphisms in genes for cytokines, chemokines, cell surface receptors, adhesion molecules, and downstream signaling

PHARMACOGENETICS

– THE CASE FOR RHEUMATOID ARTHRITIS

A particular application of gene polymorphism is the emerging field of pharmacogenetics or pharmacogenomics. This emerging and promising science is committed with the definition of how gene polymorphism may influence the several pharmacological aspects of a certain drug, including pharmacokinetics, pharmacodynamics, and side effects (Marsh and McLeod, Eichelbaum et al. 2006). A large amount of studies have been dedicated to this kind of investigation, and several clinical applications are already available.

Several anti-depressants are metabolized by the cytochrome P450 (CYP) enzymes in the liver. It has been demonstrated that polymorphism in CYP 2C19 and CYP 2D6 is associated with the metabolic rate of venlafaxine, amitriptyline, and risperidone (Eichelbaum et al. 2006, van der Weide et al. 2005). Polymorphism in these genes has been shown to influence drastically the metabolic rate and therefore the effectiveness and toxicity of these drugs. Gene polymorphism influencing the pharmacokinetics of therapeutics has been also shown to be relevant to several other conditions, such as cancer, osteoporosis, essential hypertension, and dyslipidemias (Rodriguez-Antonia et al. 2006, Ingelman-Sundberg 2006, Carbonell-Sala et al. 2006, Arnett et al. 2006, Schmitz and Langmann 2006).

Pharmacogenomics has been applied also to the management of systemic autoimmune diseases. In fact, this field is very complex and has great potential, as can be exemplified by the studies carried on in rheumatoid arthritis. Methotrexate (MTX) has been largely used for this disease and has been shown to be effective in 46 to 65% of the cases (Weinblatt et al. 2000). Toxicity of MTX leading to discontinuation of treatment in RA varies from 10 to 30% (Weinblatt et al. 1989). It is possible that gene polymorphism may be correlated to some extent with the effectiveness and toxicity of this drug in RA patients. MTX is a folic acid analog and acts as a competitive inhibitor of the enzyme dihydrofolate reductase (DHFR). Reduced folate carrier 1 (RFC-1) mediates MTX entry to cells. The efflux from the cell is mediated by the ATP-binding cassette (ABC) transporters (ABCC 1-4 and ABCG2).



TABLE III
Gene polymorphism association with susceptibility and phenotype of autoimmune diseases.

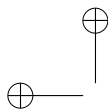
Association with susceptibility		
Gene and allele	Clinical condition	Reference
HLA-DQ2 & DQ-8	Celiac Disease	Lundin 2003
PTPN22 1858T	RA, SLE, GD, type 1 DM and JIA	Lee et al. 2007
DRB1*07 / CTLA-4 49G	Graves Disease	Kula et al 2006
GSTM1 null genotype	RA	Morinobu et al. 2006
CTLA-4*G allele	type 1 DM	Kavvoura and Ioannidis 2005
Desmoglein 1 809C allele	<i>Pemphigus foliaceus</i>	Martel al. 2002
MBL HYPA haplotype	Primary biliary cirrhosis	Matsushita et al. 2001
Association with disease phenotype		
Gene and allele	Clinical condition	Reference
CTLA-4 +49A allele	Extra-glandular Sjögren's syndrome	Downie-Doyle et al. 2006
IL4 Receptor V50 allele	Erosive rheumatoid arthritis	Prots et al. 2006
FCγRIIIB (few copies)	Nephritis in SLE	Aitman et al. 2006
ACE D allele	Macrovascular disease in SSc	Guiducci et al. 2006
IL-1α -889T allele	Lack of response to cyclophosphamide in SSc	Beretta et al. 2007
CCR5 59029G allele	Nephropathy in type 1 DM	Mlynarski et al. 2005

RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; GD: Grave's disease; DM: diabetes mellitus; JIA: juvenile idiopathic arthritis; MBL: mannose binding lectin; ACE: angiotensin converting enzyme; SSc: systemic sclerosis.

side the cell and inhibit DHFR, which reduces dihydrofolate (DHF) to tetrahydrofolate (THF), and thereby affects the cellular folate pool. MTXPGs also inhibit thymidylate synthase (TYMS), which converts deoxyuridylate to deoxythymidylate in the *de novo* pyrimidine biosynthetic pathway (Dervieux et al. 2004a). Methylenetetrahydrofolate reductase (MTHFR) is indirectly inhibited by MTX, by means of the effects of MTX on the intracellular folate pool. MTX effect on the purine synthesis ultimately increases the intracellular adenosine pool, which is a potent anti-inflammatory agent. Potentially relevant gene polymorphisms refer to genes that influence MTX transport across cell membrane and those involved in intracellular MTX pathway. RFC-1 G80A polymorphism has been associated to the effectiveness of MTX uptaken by cells. Higher MTXPG levels have been reported in RA patients with the RFC 80A/A genotype as compared with those who had the RFC 80G/G or G/A genotypes ($P < 0.007$) (Dervieux et

patients with RA (Dervieux et al. 2004b). SNPs have been reported for several members of the ABC family of transporters, some of which are involved in MTX efflux from the intracellular compartment. A recent study on the ABCB1 gene has found that patients with the 3435CC and 3435CT genotypes had a greater risk of having active RA compared with patients with the 3435TT genotype. The 3435T allele also seemed to confer a protective effect, with patients homozygous for this allele having a less severe form of RA that was more likely to respond to MTX and prednisone (Pawlik et al. 2004). Genetic variations in other members of the ABC family are quite common and shall be explored in the context of RA and MTX treatment.

The methylenetetrahydrofolate reductase (MTHFR) is the best studied of the genes in the MTX cellular pathway. MTHFR is important in the generation of 5-methyl-THF, which is the methyl donor for the methylation of homocysteine to methionine by methionine synthase (MS)



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associated with homocystein plasma concentration and seems to be associated with MTX toxicity, especially with hepatotoxicity. On the other hand, the A1298C polymorphism seems to have no effect on MTX toxicity, but seems to be associated with MTX efficacy in RA patients (Urano et al. 2002).

Thymidilate synthase (TYMS) is a key enzyme in the de novo thymidylate synthesis (conversion of dUMP into dTMP). TYMS is inhibited directly by MTXPGs and indirectly by the folate cofactor depletion induced by MTX. Two relevant TYMS polymorphisms have been studied with respect to MTX in RA patients. A polymorphic tandem repeat sequence in the 5'-untranslated region (5'-UTR) correlates with TYMS mRNA expression. Patients homozygous for the triple repeat allele (TSER*3/*3) have higher TYMS mRNA expression than those homozygous for a double repeat allele (TSER*2/*2). Another relevant polymorphism consists of a 6-bp deletion of the sequence TTAAAG at nucleotide 1494 in the 3'-UTR (3'-UTR 6-bp deletion). Apparently the 3'-UTR deletion is associated with decreased TYMS mRNA stability and expression. Preliminary evidence suggests that the TSER*3/*3 polymorphism (increased TYMS expression) lowered MTX efficacy and the 3'-UTR 6-bp deletion (decreased TYMS expression) made RA patients more sensitive to MTX (Kumagai et al. 2003).

Several other genes may affect MTX efficacy and toxicity, including AMP-activated protein kinase activator (AICAR), transformylase, γ -glutamyl hydrolase (GGH), dihydrofolate reductase (DHFR), methionine synthase (MS), methionine synthase reductase (MTRR) and serine hydroxymethyltransferase (SHMT). Eventually, the concomitant analysis of the polymorphism in several of these genes will provide an appropriate estimate of the expected therapeutic effect and toxicity of MTX in a given RA patient (Dervieux et al. 2004b).

Tumor necrosis factor (TNF) antagonists have been shown to be a rather effective therapeutic alternative for some patients with RA, Crohn's disease, psoriatic arthritis and ankylosing spondylitis. However, only up to 60% of the patients will present a satisfactory response to anti-TNF therapy. There is a functional bi-allelic polymor-

type is associated with a good response, the A/G type is associated with a moderate response, and the G/G type is associated with therapeutic failure (Gallardo et al. 2007). Although not all investigators have achieved this clear-cut association, it seems to be a prevalence among most of the studies (Ranganathan 2005).

These few examples are representative of the general trend that is quickly expanding to encompass several therapeutic agents and pathological conditions within and outside the field of autoimmunity.

PERSPECTIVES PROVIDED BY NOVEL TECHNOLOGIES – THERANOSTICS

The perspectives provided by the novel available technologies appear to promise a real revolution not only in diagnostic procedures, but also in the current paradigm for Medicine itself. The nosologic classification of diseases as we know it today may be totally reshaped in several areas of Medicine. Some fields will not undergo radical changes. For example, a patient with inguinal hernia will probably have the same diagnosis as today. In contrast, the vague diagnosis of SLE may change drastically. It may happen that we will not even talk of SLE in the future. Specific immunologic imbalances conditioned by peculiar sets of gene polymorphisms and characterized by defined patterns of immune mediators production may be identified within the broad family of patients nowadays diagnosed as SLE. Each one of these molecularly defined SLE subsets shall be associated with a peculiar clinical picture and prognosis. Accordingly, the appropriate therapy will be individualized for each one of these novel molecular-defined nosologic entities. Eventually, it is possible that efforts will be addressed to the individual molecular imbalances of each patient as opposed to the current efforts to classify patients into pre-defined general nosologic categories. This trend has been clearly announced as the Individualized Medicine.

On the top of that, pharmacogenetics will provide an additional degree of individualization for therapy. Once the molecular imbalance is defined and the appropriate therapeutics is devised, the physician will



TABLE IV
Fiction patient in year 2097.

• Disequilibrium Th1/Th2 in peripheral blood → 65%
• Depressed proportion of Treg in peripheral blood → 0.5%
• DRB1 gene polymorphism → presence of single copy of *0404 allele
• MBL gene heterozygosity → presence of alleles A and D
• TNF- α gene promoter polymorphism at -308 → allele -308A in double copy
• Homozygosity for allele 131R in Fc γ RII gene
• Protein tyrosine phosphatase non-receptor 22 (PTPN22) → single copy of 1858T allele
• Decreased Thymus Recent-Emigrant T cells → 15,4678 TREC copies/ μ g CD4 \oplus DNA
• Hyperexpression of IL-17, IL-2 and osteoprotegerin
• Acceleration in IFN- γ -induced gene activation sequence in macrophages

for some applications it is already feasible to assess how polymorphism of individual genes influences the therapeutic response to certain drugs. It is quite possible that the molecular definition of disease condition and therapeutics on an individual basis will occur concomitantly in an interactive fashion. This trend has been termed Theranostics and may dominate the management of patients in the future (Picard and Bergeron 2002, Jain 2002).

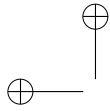
MEDICINE IN YEAR 2097

It seems not risky to forecast that Medicine will change drastically in the coming decades. Many of the clinical entities, diagnostic procedures, and therapeutic options of today shall disappear. Novel paradigms, the emerging technology, and the evolving clinical practice shall re-shape each other towards a more individualized Medicine. In a provocative scenario, one can foresee the clinical workout of a patient in year 2097 (Table IV). This may cause wonder and skepticism to some of us nowadays in the same way that our current medical approach would have caused if shown to a 1597's physician (Table I). This historical perspective is reassuring in that it lends credibility to the viability of an accomplishing future for Medicine. However, a word of caution must be said with respect to the economical impact of the current and evolving health care. The constant progress in medical diagnosis and treatment has generated genuine cost inflation in the health care system

come economically feasible in order to thrive.

RESUMO

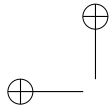
O homem tem buscado sucessivas abordagens para o entendimento e manejo das doenças. Partindo de conceitos sobre-naturais e procedimentos místicos, uma abordagem científica vigorosa vicejou com base em disciplinas fundamentais como a anatomia, microbiologia, bioquímica, fisiologia, imunologia, patologia e farmacologia. O conhecimento integrado resultante contribuiu para a atual classificação das doenças e a forma com que a Medicina atual é praticada. Apesar deste considerável progresso, esta abordagem é insuficiente quando se trata de condições inflamatórias sistêmicas, como o lúpus eritematoso sistêmico, que abrange condições variando de formas brandas e pauci-sintomáticas até condições rapidamente fatais. O tratamento dessas condições é frequentemente insuficiente e novas abordagens são necessárias para progresso adicional nessas áreas da Medicina. Um avanço recente foi obtido no que tange às síndromes auto-inflamatórias hereditárias, nas quais a dissecação molecular dos defeitos gênicos subjacentes forneceu direcionamento para terapia orientada a alvos moleculares específicos. Esta abordagem é passível de aplicação às doenças auto-imunes sistêmicas com a compreensão de essas condições podem ser consequência da interação de estímulos ambientais específicos e uma gama de vários polimorfismos gênicos interconectados. No escopo dessa transformação, a aplicação dos princípios de farmacogenéticas poderá contribuir para o progressivo desenvolvimento de uma Medicina personalizada vigorosa



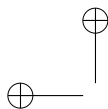
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