Unravelling the Role of Infectious Agents in the Pathogenesis of Human Autoimmunity: The Hypothesis of the Retroviral Involvement Revisited

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Abstract: The incidence of autoimmune disorders is increasing worldwide. Several theories have been proposed to explain how the breakdown in the balance between autoregulatory immune pathways and pathogenic autoreactivity generates autoimmunity. On the basis of a large body of epidemiological, clinical and experimental evidence, it has been suggested that an unfortunate interplay of genetic susceptibility and environmental factors must play an important role in generating the abnormal autoimmune response. Although genetic factors have been well dissected, the environmental agents that may be causative of disease are still under investigation. Their discovery is obviously relevant because they enable us to devise preventive and therapeutic strategies in trying to halt the progress and ultimately treat this category of disorders. Among the environmental factors, infectious agents have been proposed as the best candidate triggers in the autoimmune pathogenesis. The observation that a long preclinical period often precedes the clinical onset of disease, in analogy to the clinical symptoms of AIDS, led to propose exogenous and endogenous retroviruses as suspected culprits for organ and non-organ specific autoimmune disorders. The hypothesis is revisited in this article in the light of our research experience over the past years and of relevant literature emerging in the field.

Keywords: Retroviruses, endogenous retroviruses, exogenous retroviruses, autoimmune disease.

INTRODUCTION

The incidence of autoimmunity is increasing worldwide [1]. Several disorders are recognised as autoimmune and generally classified according to the organs or tissues that are affected [2, 3]. In autoimmune conditions the breakdown in the balance between autoregulatory immune pathways and pathogenic autoreactivity leads to aggressive antibody and T-cell mediated reactions directed against antigens expressed by the host's own tissues.

Throughout the years, a remarkable number of theories have been proposed to explain how the immune system recognises self. On the basis of a large body of epidemiological, clinical and experimental evidence, it has been suggested that an unfortunate interplay of genetic susceptibility and environmental factors must play an important role in generating an abnormal immune response [4]. Most of the common autoimmune diseases are polygenic in nature; in any given individual afflicted with the disorder, multiple genetic defects may contribute together to the autoimmune process. Studies related to the past decade report a concordance rate of approximately 25% in monozygotic twins for the most common autoimmune disorders, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), insulin-dependent diabetes mellitus (Type 1 diabetes, T1D) or multiple sclerosis (MS) showing that genetic factors influence the development of autoimmunity. Alternatively, approximately a 70% discordance rate emphasizes the importance of environmental factors [5]. As a consequence, the discovery of environmental factors causing autoimmunity would obviously become relevant, thus enabling us to devise novel therapeutic measurements for trying to halt the progress and ultimately cure these disorders.

Among the environmental factors, infectious agents have been proposed as the best candidate triggers in the autoimmune pathogenesis. Nevertheless the interaction between infectious agents and the host and the relationship with autoimmunity is complex. In addition to the identification of specific triggers or precipitators of autoimmunity, the accelerator or hygiene hypothesis have also been postulated: cleaner living conditions would lead to enhanced incidence of autoimmune disorders, asthma and allergies [6]. Some infectious agents may exert a protective role, by modulating the immune system [4]. Underlying pathogenetic mechanisms can be aspecific or 'bystander' or more specifically related to certain infections. As a general effect, infectious agents may enhance antigen presentation of self-antigens, cause the involvement of different self antigens or of different epitopes of the same autoantigen ('epitope spreading'). As a consequence, during the chronic state of disease, autoreactive T cells can be challenged by an increased number of autoantigenic peptide determinants which may be responsible for the different clinical phenotype of the disorder over time [7].

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Infections can also produce apoptosis, or programmed cell death; this phenomenon plays a central role in the maintenance of self-tolerance and the homeostatic control of lymphocyte populations including autoreactive T and B lymphocytes [8]. Whenever the removal of apoptotic cells is defective, secondary necrosis may follow apoptosis; thus nuclear material is accumulated within affected tissues generating a survival signal for autoreactive immune cells.

The understanding of autoimmune diseases experienced an impressive boost, since toll-like receptors (TLRs) have been identified [9] as possible key players in the pathogenesis. This group of Type 1 transmembrane proteins is growing in size and currently there are at least 13 TLRs identified in mice and 11 in humans [10]. Most of these TLRs are extracellular, except for TLRs 3, 7, 8, 9, which recognize intracellular signals. TLRs signal through two main pathways, ultimately to activate NF-kB in the nucleus. Most of the TLRs use the adaptor protein MyD88; instead, TLR3 uses a different adaptor, the protein TRIF (Fig. **1**). TLRs engaged by pathogen-associated molecular patterns (PAMPs) derived from viruses, bacteria and fungi play an essential role in the early and prompt activation of immune cells including antigen-presenting cells, T cells and B cells. Some TLRs in humans recognise damage associated molecular patterns (DAMPs) derived from apoptotic or necrotic cells; this explains the relevance of microbial DNA and RNA in triggering innate responses to pathogenic microorganisms. Activation of antigen-presenting cells (APC) through TLRs triggers induces early upregulation of MHC class I and class II and co-stimulatory molecules that are critical for full-activation of T cells. The pathways lead to the production of inflammatory cytokines including IL-6, IL-12, IL-18, IFN-γ and TNF-α [10]. Subsequently, it has become clear that T cells express TRLs and thus can be directly stimulated by their ligands [10]. In addition to the activation of immune cells that directly participate in infections, the stimulation through TLRs can also have an effect on regulatory T cells.

By analogy to the adaptive immune system, the innate immune system requires mechanisms for self-nonself discrimination. Nevertheless, the distinct recognition between nucleic acids of mammalian versus those of microbial origin by TLRs is imperfect. Endogenous ligands, such as RNA and DNA can activate TLRs under certain conditions and induce a typical autoimmune reaction. In addition, certain DNA-binding molecules [11] that bind both exogenous as well as endogenous DNA, released from dying or necrotic cells, can facilitate endocytosis. In providing

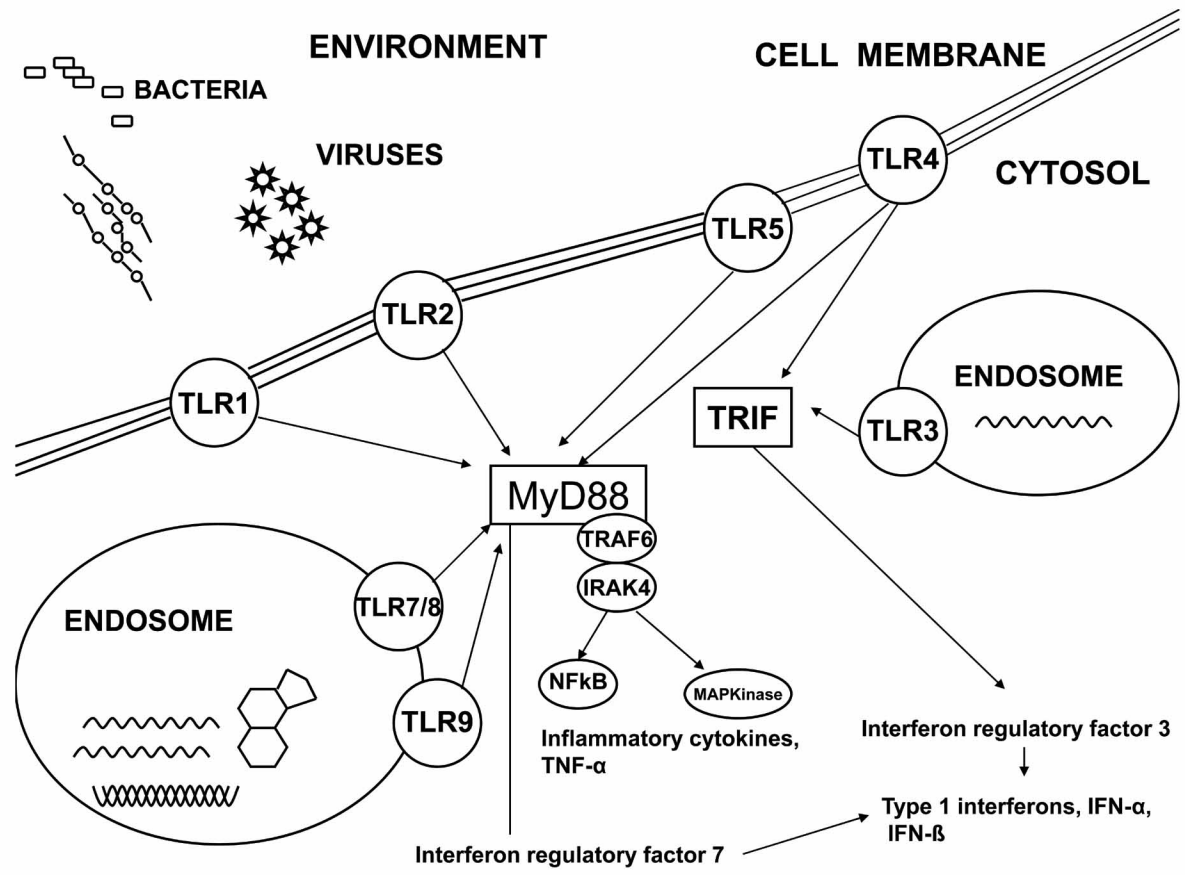

Fig. (1). Signalling pathway of Toll-like receptors. The figure reports the intracellular and extracellular location of Toll-like receptors and their intracellular cascades (modified from Wong and Wen 2008 [10]).

examples, SLE is characterised by the development of IgG serum autoantibodies directed against nuclear antigens such as DNA, nucleosomes and ribonucleoproteins (RNP). Genetic predisposition leads to the development of IgG2a autoantibodies, accumulation of IgG2a-containing immune complexes in the kidney, development of nephritis and increased mortality in animal models [12]. MyD88 signalling and especially the role of nucleic acid-specific TLRs, such as TLR-9 and TLR7 have been analysed. Self-reactive B cells are activated in the periphery after BCR-dependent internalisation of nucleotide containing self-antigens and subsequent co-activation of intracellularly expressed TLRs. Conversely, deletion of MyD88, the common signal transducer of TLRs except TLR3, in FcgammaRIIB-/- B6 mice, abolishes the development of IgG2a and IgG2b autoantibodies and prevents nephritis [13-15].

Autoimmune encephalomyelitis (EAE) can be induced by injection of myelin oligodendrocyte glycoprotein (MOG) peptide emulsified in complete Freund's adjuvant and pertussis toxin to open the blood-brain barrier [12]. Again, MyD88-deficient mice, do not develop EAE, do not generate MOG-specific Th17 cells and fewer Th1 cells as compared to controls, indicating that induction of disease is dependent on TLR signalling.

In Bio-breeding diabetes-resistant (BB-DR) rat model, development of diabetes can be triggered by infection with the Kilman rat virus (KRV), a single-stranded DNA parvovirus [10, 16]. The virus does not directly infect the pancreas, instead alters the balance between pathogenic and regulatory cells, reducing the CD4+ $CD45RC^{low}$ subset. The disease expression can be increased by stimulation with TLR ligands in addition to the virus infection. TRL ligands include lipopolysaccharide, CpG oligonucleotides, zymosan and poly I:C.

Among the antigen-specific mechanisms 'molecular mimicry' [17], the disclosure of novel antigenic epitopes or the so called 'superantigen effect' can be encountered. The basic concept in molecular mimicry is that foreign proteins may have sequence similarity with self-antigens, therefore initiating cross-reactivity [4]. The immune system can evoke activation of naïve autoreactive T cells specific for the self-molecule.

According to another theory, self proteins may undergo post-translational modifications, denaturation, and misfolding leading to autoimmunity [7]. Also cryptic antigens, physiologically sequestered in organs, can subsequently be exposed to autoreactive T lymphocytes as in the course of several infections.

Superantigens are produced by a variety of microorganisms, including bacteria, mycoplasma proteins or virus-infected cells [18]. They are potent immunostimulating molecules because they have the unique ability to polyclonally activate a large number of T lymphocytes through binding between class II major histocompatibility (MHC) molecules on antigen presenting cells and the variable Beta chain (Vβ) of the T cell receptor, independently of its antigenic specificity. Conversely, conventional antigens clonally activate only a small proportion of T cells by binding to MHC class II molecules. They both include exogenous retroviruses (Ex-RVs) and endogenous retroviruses (E-RVs).

The observation that a long preclinical period often preceds the clinical onset of the autoimmune disease, in analogy to HIV infection, led to hypothesise that retroviruses could be responsible for organ and non-organ specific human autoimmunity [19]. This hypothesis is here revisited in the light of our research experience over the past years and of relevant literature emerging in the field.

EXOGENOUS RETROVIRUSES AND AUTO-IMMUNITY

ExRVs are a large family of enveloped animal viruses, with single stranded positive sense RNA genomes [20]. The defining characteristic of Ex-RVs is that, following infection of a target cell, the genomic RNA is converted to a double-stranded DNA, becoming stably integrated into the chromosomal DNA of the host cell. This DNA provirus then replicates by directing the synthesis of the viral proteins, by using cellular mechanisms for transcription and translation. Originally they were divided into three general subfamilies: oncoviruses, subdivided into A-, B-, C-, and D-types on the basis of morphological appearance by electron microscopy (EM) [21, 22], that cause tumours and immuno- deficiency diseases, lentiviruses, or slow viruses, that cause chronic progressive disease, and foamy viruses (spumaretroviruses) that cause vacuolisation of cells in culture, but has not been demonstrated yet to produce disease *in vivo*.

Initial demonstration of the possible involvement of RVs in the pathogenesis of autoimmunity came from animal models [23-25]. Particles of slow replicating viruses were demonstrated by EM in pancreatic islet beta cells of the non-obese diabetic (NOD) mice [23, 24]. Subsequently elevated levels of 2'-5'-oligoadenylate synthetase anti-viral activity were detected in the cytosol of thyroid epithelial cells of the obese chicken (OC) [26], an animal model for Hashimoto's thyroiditis, and of pancreatic beta cells of BB rats, another strain which spontaneously develops a Type-1 like diabetic syndrome [27, 28].

Researches originally tried to unravel the involvement of RVs in the pathogenesis of human autoimmunity, by searching for them in thyroids of thyrotoxic patients with Graves' disease (GD). The strategy implemented was that of searching for 'molecular footprints' of a known EX-RV (i.e HIV, HLTV, and foamy virus), which may have shared sequence homology with the one suspected to be involved in autoimmunity. HIV-like sequences were first revealed by Southern blotting in DNA of GD thyroids [29]; unfortunaterly these results were not reproducible

[30, 31]. However, despite the initial unsuccessful attempt, the same approach stimulated a great deal of interest in other laboratories. Subsequent experiments indicated that DNA prepared from peripheral blood lymphocytes (PBL) of patients with GD and/or T1D produced positive signals after hybridisation with HTLV-1 sequences [31]. However, this time, we were unable to confirm these findings (unpublished observations). Subsequently, gag proteins of the human foamy virus were detected by indirect immunofluorescence (IFL) in thyroid follicular cells again of patients with Graves' disease [32]. DNA sequences related to the same human RV could be detected, by both Southern Blotting and PCR, also in PBL of the same patients [33]. However, as in previous circumstances, other laboratories have failed to reproduce these initial data[1] [34, 35].

Positive findings were also reported, when PBL of patients with MS were tested, in another set of experiments, with the same human foamy virus and by the same molecular biology approaches [36]. These results are still lacking confirmation. However circulating antibodies reacting with proteins of the test virus were not detectable in patients in the mentioned studies.

RVs and systemic autoimmune diseases, such as RA [37] and Sjögren syndrome (SS) also attracted attention. Goats infected with caprine arthritis encephalitis virus developed inflammatory arthritis [38]. In addition, HTLV-1, in areas like Japan where is endemic, has been associated with a chronic oligoarticular arthropathy and symptoms resembling to those present in rheumatic disorders suh as SS [39] and polymyositis [40], while HIV-1 has been associated with symptoms of SS [41]. However, studies on both RV diseases have shown that only 1 to 5% of infected individuals develop an autoimmune like syndrome, indicating that RV-induced autoimmunity is a rare reaction to known RV infection. Other autoimmune diseases reported in the setting of HIV infection include anti-phospholipid syndrome, autoimmune thrombocytopenia, vasculitis, GD and primary biliary chirrosis (vide infra) [42]. Many theories for the induction and mantainance of such responses have been entertained including molecular mimicry between HIV proteins and self molecules, CD4+ T cell loss accompanied by loss of normal immune regulation that dictate self and non-self reactivity, and defective negative/positive selection of T cells [43]. It has been demonstrated that RV proteins may have a direct effect on the immune system: human synovial cells which are transfected with the transactivator gene of HLTV-1 termed tax, showed enhanced proliferation and production of proinflammatory cytokines [44]. Similar findings were obtained *in vivo* in mice transgenic for HTLV-1 tax gene, which develops an erosive polyarthritis resembling RA [45]. Returning to human autoimmune diseases, patients with SLE and SS were shown to have antibodies cross-reacting with RV group specific antigen (gag) proteins [46, 47], but in the absence of human, molecular and clinical evidence of HIV-1, HTLV-1 or antibodies to other known RVs. Furthermore, monoclonal antibodies to known exogenous RVs stained synovial cells of patients with RA or submaxillary glands of patients with SS [48]. More recently, human foamy virus bel 1 sequence was detected by RT-PCR in patients with autoimmune rheumatic diseases [49]. Antibodies against bel 1 and pol foamy virus sequences were also depicted.

In order to further disclose the putative involvement of RVs in the aetiopathogenesis of human autoimmunity, co-culture techniques were introduced. Thus, Garry *et al.* found that tissue homogenates prepared from biopsies of salivary glands of patients with SS and co-cultured with the human T cell line H9 [47], were able to infect it, as identified by EM, with intra-cisternal A type particles antigenically related to HIV (HIAP-1) revealed by EM. It was suggested that these particles were transmitted endogenously within the human genome and exogenously as defective particles. The same co-culture approach was then applied to RA. In a subsequent study, virus-like particles were detected in 5 out of 8 H9 cell preparations, two weeks after incubation with synovial fluid from patients with active RA [50, 51]. We co-cultured H9 cells not only with thyroid homogenate, as it was done in SS, but also with viable human thyrocytes obtained from the affected glands. We were unable to identify the transfer of RV particles to the H9 T cell line [52, 53].

Subsequently, over 90% of patients with SS or SLE and over 85% of patients with GD [54, 55] were shown to present serum antibodies reactive to HIAP-1 associated proteins, compared to only a minority of disease controls and healthy individuals. In patients with GD, the association between the presence of anti-HIAP1 antibodies and HLA susceptibility was, apparently, highly significant [55]. Although we are still lacking from confirmation of these data, anti-RV antibodies were reported also in patients with primary biliary cirrhosis (PBC) [56]. Nevertheless several laboratories failed to confirm the disease specificity of the phenomenon, because indeterminate RV seroreactivity was detected also in a high percentage of normal individuals [57, 58].

Viral particles associated with reverse transcriptase activity were successfully isolated from cultures of choroid plexus cells obtained post-mortem and from EBV-immortalised PBL of MS patients [59], but not from non-MS donors. Sequence alignment of the MS-associated retrovirus (MSRV), with other retroviruses revealed a phylogenetical relation to C-type group of Oncovirinae. MSRV sequences were subsequently detected only in untreated MS patients, but not in those treated with immunosuppressive therapy. The implication of MSRV as a pathogenic retrovirus in MS,

[1]Roura-Mir, C., Musany, W., Sospedra, M., Catálforno, M., Pujol-Borrell, R., Jacaquerada, D. and Flűgel, R,M. Absence of human spumaretrovirus (HSRV) related message or sequences in cells from Graves' disease patients. Abstracts of the 12th European Immunology meeting, 1994 14-17 Jun; Barcelona, Spain. P. 359.

would also give a clue to the role of Herpesviruses, repeatedly implicated in the pathogenesis of MS. Since Herpesvirus type 1 immediate early proteins can transactivate MSRV expression *in vitro*, it was postulated to act as triggering cofactor of MSRV in MS pathogenesis.

Lately, a novel infectious RV, human retrovirus 5 (HRV-5) has been linked to the pathogenesis of rheumatic disorders [60]. Proviral DNA of this virus was detected in approximately 50% of synovial samples of arthritic joints and in over 10% of the blood samples of patients with RA and SLE. However, HSRV-5 appears to be found with a very low genome copy number in the most normal tissues. Still it remains to be explained, whether HRV-5 is aetiologically important in these diseases.

If controversial are the data on the evidence of Ex-RV in autoimmunity, the mechanisms also remain unknown by which RVs may induce autoimmunity. Several hypotheses have been proposed: molecular or antigenic mimicry between ribonucleoprotein antigens and viral core proteins has been suggested to account for the anti-RV antibodies commonly found in the patients with SLE and SS [61, 62]. The coordinate interaction between infectious exogenous viral particles and ERV of the human genome has also been hypothesized. In providing examples, the *tat* gene of HIV-1 stimulates expression of HRES-1/Rab-4 protein *via* transactivation of HRES-1 LTR (vide infra) [63-65].

ENDOGENOUS RETROVIRUSES AND AUTO-IMMUNITY

ERVs are permanently integrated sequences. It is estimated that HERVs constitute about 40% of the human genome [5]. Phylogenetic studies have shown that some HERVs emerged over 3 million years ago, whereas others appeared after the divergence of hominoid and ape lineages [66][2]. Consequently HERVs have been present in our genome for a long time. They are also named 'fossil viruses' because they represent footprints of ancient retroviral infections of the human host. In general, ERVs have found a safe ecological niche within their human hosts and, in contrast to exogenous (Ex-) RVs, they are transmitted vertically through the germline as stable inherited Mendelian traits.

Over millennium, ERVs underwent multiple amplifications and transpositions, therefore, multicopy or single copy proviruses are distributed along cell DNA [48, 67b]. Regarding their genomic organisation, ERVs are similar to present day exogenous retroviruses, such as HIV and HTLV. They are constituted of gag, pol and env regions located between two long terminal repeats (LTRs) that are fundamental in regulating gene expression [67b]. As exogenous retroviruses gag and env-genes encode retroviral capsid and envelope proteins, pol genes encode enzymes for viral replication, integration and protein cleavage. Retroviruses are retrograde in the sense that the flow of genetic information is reversed compared with the normal pathway of molecular biosynthesis DNA-RNA-protein. All retroviruses necessitate the conversion of viral RNA into an intermediary cDNA, catalysed by reverse transcriptase. Nowadays classification and nomenclature of ERVs are controversial [67a]. Originally HERVs were classified into 3 broad classes based on sequence comparison with animal retroviruses [67b].

Class I HERVs are subdivided into 6 groups, having homology with infectious mammalian type C viruses. 3 families within this class share similarity with murine leukaemia virus (MuLV) and baboon endogenous virus (BaEV) in the highly conserved pol, gag, and env regions. Class II HERVs included 10 groups and showed homology with mammalian Type B (i.e. MMTV) and Type D retroviruses. HERV-K is biologically the most active human endogenous retrovirus family. Foamy viruses-related HERVs were classified as class III HERVs, including the solitary representative HERV-L.

Although it is impossible to trace back their putative ancestral biological functions [48], HERVs most likely underwent complete endogenisation; thus, from a speculative point of view they probably have been retained because some of them may have conferred also biological benefits to the human host or exerting some useful biological functions [67a]. Alternatively, it is possible to speculate that some ERVs during evolution have been difficult to be eradicated and thus persisted. In providing examples, HRES-1/1 entered the genome in primates, presumably as an exogenous retrovirus. This was detected and cloned from a recombinant lymphocyte DNA library based on cross-hybridisation with HTLV-I LTR and gag region-containing probe [68]. HERV-R (ERV3) is highly expressed in trophoblastic cells and results in high concentrations of env protein in syncytiotrophoblasts [69]. It has been suggested that it plays a role in protecting the developing fetus from maternal immune responses. It is possible that the ERVs may change the pattern of gene expression during embryogenesis, thus altering different rates of development of different parts of the embryo. It has even been postulated that they may protect their hosts against infections by closely related Ex-RVs, thereby contributing to their eradication. Antiviral resistance may be conferred by retroviral receptor blockade (by HERV products) and interference of replication through antisense mRNA. For the presence of similarities between HIV cytotoxic T lymphocytes peptide sequences and regions of HERV-K10, it has been suggested that in some cases previous exposure to HERV peptides could potentially immunise certain individuals [70].

There is a general consensus that some ERVs were expressed as full-size transcripts and others as transcripts of varying size at RNA level, mostly in

[2]Roden, D., Ejtehadi, H.D., Rowland-Jones, S. and Nelson, P.N. Use of bioinformatics to highlight antigenic regions of exogenous and endogenous retroviruses. Immunology 2001; 104:S112-113.

tumour cells/cell lines and placenta [76], but also in certain normal tissues [77]. However, it can be predicted that few E-RV transcripts are translated, because most are truncated and/or their open-reading frames (ORFs) are interrupted by termination codons or frame shift mutations. Their proteins are rarely expressed and if they are, their functions are unknown [78] and it is very seldom that they packed in particles, the ultimate characteristic of a fully expressed RV. In further supporting the 'quiescent concept' at least in humans, although ERVs may potentially rearrange the genome by recombination and transposition, it is unusual to see them cooperating in the regulation of cellular gene expression or that of adjacent cellular genes, as known in one example for the amylase gene [79], ZNF80, Cytochrome C1, Kruppl-like H-plK, phospholipase A2-L in humans [78]. This is because retroviral genes integrated in the genome are bordered by short direct repeats of host DNA and LTR sequences [78]. These LTRs can influence the neighbouring genes because they may contain transcriptional regulatory elements, such as enhancers, promoters, hormone responsive elements and polyadenylation signals.

Several arguments support the possible involvement of HERVs in the development of malignancy. HERV mRNA, functional proteins or retroviral-like particles have been implicated in tumourigenesis [80]. They may be also associated with the generation of new promoters or the activation of proto-oncogenes. In providing examples, supporting the putative involvement of HERVs in malignancies, HERV-R mRNA is overexpressed in small-lung cell carcinoma [80]. A teratocarcinoma cell line has been shown to possess a HERV-K sequence and to secrete retroviral-like particles [81].

Proteins of the HERV-K family of HERVs were detected in testicular germ cell tumours (TGCTs) in patients producing an immune response to gag and env proteins [82, 83]. It has also been demonstrated that HERV may contribute to encode immunosuppressive proteins [78, 84], therefore, HERV proteins have been proposed as targets of antitumour immunotherapy. It has also been postulated that HERV-K is involved in the pathogenesis of human breast cancer [85, 86]. HERV type C is inserted into the growth factor gene pleiotrophin (PTN), which appears to be responsible for the aggressive and invasive growth of human choriocarcinoma [87]. Further studies are necessary in order to unravel and elucidate the putative determinism of HERVs in human tumourigenesis and cancer.

In reference to autoimmunity it must be emphasised that large numbers of ERV sequences are found multiply integrated in loci, regulating the production of key molecules responsible for efficient immune responses (e.g. histocompatibility antigens), but again they are apparently not translated [88]. In this context, it has to be emphasised that ERVs (HERV-W, HERV-H) and MS have been polarizing scientific attention in trying to link them to its pathogenesis [89]. Although we question, whether MS is strictly an autoimmune disease (it is still difficult to detect in these patients clear autoantibody responses, or T cell reactivity against relevant mielin related autoantigens), the data on ERVs remain quite encouraging.

Polymorphisms of the ERV have been linked to lupus [71]. The HRES-1 genomic locus is prone to somatic mutations due to homonucleotide repeats [71] and was found transcriptionally active in lymphoid cells, melanoma cells and embryonic tissues [68]. The locus encodes a 28kD nuclear autoantigen recognised by lupus autoantibodies. Sera of patients with MS, progressive systemic sclerosis, SLE and SS contained significantly higher HRES-1 peptide binding activity that of sera of normal donors [72-74]. The anti-sense strand codes for a small GTPase HRES-1/Rab4 that modulates surface expression of CD4, thus infection of T cells by HIV-1 [63] and influences T cell activation in LES [75].

Regarding T1D, an ERV (HERV-K IDDM22) was identified in PBMC of patients [90] but at least 9 groups [91-99], in trying to reproduce the original data immediately since the paper came out, did not succeed. The issue of the possible involvement of ERVs in inflammatory vascular disease was also raised [100].

Animal models were also implemented to improve our understanding of HERVs. ERV particle formation was detected in islets of non-obese diabetic (NOD) mice [24], but apparently not in the islets of human 'diabetic' pancreases, or in any other human tissue affected by autoimmunity (including that of MS patients). Yet again, ERV-like particles assemble, when cell lines are established from diseased tissue (e.g. in MS), or are co-cultured with other cell lines (e.g. in SS) [48].

In a lupus model, an 8.4 Kbp endogenous retroviral transcript was found expressed in affected mice [101]. A lupus-like autoimmune disease was generated in MRL-lpr/pr mice [102] in which a retroviral element was introduced in one of the introns of the fas apoptosis gene altering the splicing of fas transcripts.

Mechanisms, whereby HERVs could influence autoimmunity include molecular mimicry, superantigen motifs, aberrant expression of antigens or neo-antigen formation due to HERV and/or exogenous viral combination. Experimental evidence also suggests the possibility that ERVs are the precipitating factors instead of triggers in the generation of autoimmunity [48]. Several reports demonstrated RV immuno-suppressive effects on lymphocytes and monocyte-mediated functions both *in vivo* and *in vitro* or causing imbalance in Th1 and Th2 responses. In a theoretical cascade of events, ERV expression can be induced by several exogenous and/or endogenous factors, such as B and T cell mitogens, hormones and cytokines. Transcription and translation of core protein p73 of RV intracysternal A type particles (IAP) are, for example,

inducible by glucose in cultured islet beta cells and an increase in IAP expression has been observed in beta cells of hyperglycaemic, genetically diabetic (db/db) mice. In addition, molecular mimicry between insulin and retroviral antigen p73 has been suggested, because two diabetes-susceptible inbred strains, which constitutively express the IAP genome in beta cells, spontaneously develop insulin autoantibodies that cross-react with p73.

Experimental data support the distinct possibility that ERVs and exogenous viruses interact to lead to autoimmunity [48]. Supertransfection with heterologous DNA can enhance RV expression or trigger latent RV expression. A mini-chromosome might serve as an insertional target for RVs, generating novel hybrid viruses with different biological properties, as suggested for EBV in MS. A pathway of interactions between exogenous RNA viruses and ERVs has also been recently proposed [103]. For example, endogenous reverse transcriptase can, in a host-virus-specific manner, generate cDNA transcripts from lymphocytic choriomeningitis virus (LCMV), as it infects cells *in vitro* and *in vivo*, and that this cDNA persists long after the original acute infection has waned below detectable levels. Interestingly, LCMV infection was originally shown to up-regulate ERV replication [103]. It was also speculated, whether Coxsackie B3 itself both in dilated cardiomyopathy (DCM) and in T1D, could act as a superantigen or activate an ERV remains speculative for the moment [104].

In future, we believe that animal models and multicentre patient studies will need to be planned to unravel the links between specific HERVs and autoimmune diseases; indeed many HERVs are also expressed in varying amounts in normal tissues [105, 106].

PERSONAL CONCLUSIVE VIEWS

Despite the undeniable advances in the field of immunology, the reason behind the loss of immunological tolerance against self, thus leading to an autoimmune disease, seems to be still confined to the philosophical sphere. Throughout the years, the number of theories thrown into the scientific arena has been quite remarkable; nevertheless we are still far from approaching the solution. There is a general consensus that the environment plays an important role in the development of autoimmunity in individuals who are genetically predisposed to disease. As best exemplified by the T1D model, for long-time it has been suggested that viral infections may play a role in the development of disease. Nevertheless it remains unclear how the different hypothesised pathogenetic mechanisms (vide supra) intervene in the disease process. In most cases, T1D does not occur as a result of direct infection of the pancreas [10]. Antigenic molecular mimicry is another mechanism that is often hypothesised for the development of autoimmune disease in the context of infection. However, there is relatively little evidence for this in T1D [107]. The BB-DR rat [16] is an additional example of how an autoimmune disease can be precipitated by viral infection. The KRV does not directly infect the pancreas, affecting instead the balance between pathogenic and regulatory T cells. In addition, the disease expression can be increased by the concurrent innate immune system activation by multiple TLRs agonists (i.e. bacterial infections) in temporal proximity to the viral infection. In searching for what might cause human autoimmunity, retroviral superantigens have certainly appealed investigators for a series of reasons not least that RVs, when activated, can affect several immune functions and, perhaps more relevantly, that known RV infections in humans are characterised by a long preclinical period before overt disease onset, as best exemplified by HIV infection, similarly to what occurs in human autoimmune diseases, as best exemplified by T1D [19]. The only difference between the two just mentioned pathological situations is that in AIDS the RV has been identified, in T1D it has not. Thus, if the involvement of RVs in human autoimmunity theoretically makes sense, why then is so difficult to identify them? Many examples of positive and sometimes negative associations between infectious pathogens and autoimmune disorders have been reported. Nevertheless clear proof for any given culprit has been difficult to establish because of the fact that clinical manifestations appear heterogeneous in individual autoimmune diseases. In addition, especially in reference to retroviruses, the scientific community has been confronted by; 1. Claims which could not be reproduced by others; 2. The difficulty to understand not only why certain laboratories have not pursued path they initially opened, and certainly looked quite promising; and 3. Why did other laboratories not try to reproduce or expand certain findings originally produced by others. Furthermore, most epidemiological studies were unable to detect exposure/disease associations, were not prospective or long-term, did not start in infancy, had imprecise or infrequent exposure estimates or failed to account for genetic susceptibility. Several explanations can be addressed to explain the difficulties encountered in identifying the etiological agents in autoimmunity. One reasonable hypothesis is that triggering infection generally occurs many years before the clinical onset of autoimmune disease. First of all, it is reasonable to believe that triggering infection heals rapidly so that its virological and serological footprints have disappeared by the time of clinical onset of the autoimmune disease.

In experimental animal models, sometimes all looks quite simple and straightforward and this is quite remarkable, for example, RV like particles were observed by EM in the islets of NOD mice. Nevertheless, similar evidence has not been produced with human pathological material affected by autoimmunity, not only with ‘diabetic’ islets, but also with autoimmune thyroids, a tissue much more easily obtainable. It is also remarkable that scientists who induced or enhanced RV expression (as in established cell lines obtained from human tissues affected by

autoimmunity) and managed to express relevant RV proteins, did not take advantage of these RV products and try to see whether they were causing the equivalent disease in animals.

Regarding the 'superantigen' theory, this still lacks confirmation; the supporting knowledge is still limited to the evidence of TCRVβ restriction in some isolated autoimmune conditions. These include the peripheral blood T cells of T1D patients [108] and T cells infiltrating thyroids affected by autoimmunity [109, 110]. Contrasting data are also reported regarding the predominant CD4 or CD8 population of lymphocytes infiltrating the islets of newly diagnosed diabetic patients [90, 111]. In addition, different findings were reported in reference to the TCR pattern in the same insulitis process. In fact, a skewed TCR Vβ7 was detected in the pancreases studied in Pittsburgh [111], no TCR skewed pattern was observed in similar pancreas studies in Barcelona [112], or Vβ8 positive T cells were instead over-represented in the gland investigated in Turku [113]; on the opposite, in pancreatic biopsies taken from similar patients in Osaka, dominant TCR alpha-chain clonotypes were expressed in the insulitis process [114].

On the basis of a large body of epidemiological, clinical and experimental evidence reported so far, we believe nowadays, that the relation between infections and autoimmunity is more complex and multifacet than initially thought, even in relation to the fact that clinical manifestations appear heterogeneous in individual autoimmune diseases. In addition, during lifetime, encountering multiple related or unrelated infections may lead to autoimmunity. The 'inflammatory history' of an individual may be characterised by subsequent expansion of a pool of both un-related non autoreactive but also autoreactive memory T cells.

It is also possible that similar viruses in general may have the ability to affect the outcome of an autoimmune disease. Therefore, it becomes evident to be more appropriate to use the term 'viral profiles' rather than viral types. As a consequence different viral types may share identical 'viral profiles' in relation to autoimmunity symptoms. According to this modern view the effect of a viral infection on an autoimmune disease will be the consequence of the 'specific viral profile' acting in the context of the host specific 'autoimmune status' at the time of infection [115]. In conclusion, many viruses could be responsible for predisposing individuals to autoimmune disease on the basis of their genetic background. Common mechanisms among different viruses rather than one single culprit may underly the multifacet relation between environment and penetrance of disease. Identical viruses could also either induce or prevent autoimmune diseases depending on the 'autoimmune state' of the infected host. Similarly, according to this theory, different viruses could be responsible for the autoimmune process to be prevented, to develop or to be halted.

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