Innate immunity in systemic lupus erythematosus: Sensing endogenous nucleic acids

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\textbf{Abstract}

Historically, the involvement of complement – an integral part of the innate immune response - in the pathogenesis of lupus was recognized early. Emphasis shifted quickly however to the specific immunity with scientists concentrating on the adaptive immune response (autoantigens, auto-reactive T cells and autoantibodies). Similarly, the detection of interferon alpha (IFN\textsubscript{a}), another key mediator of innate immunity, in the sera of active lupus patients by Hooks and Moutsopoulos in 1979 was poorly understood and thus ignored for many years. More recently however, the realization that a) endogenous ligands (“stressors”) derived from a “stressed” host can be potent inducers of inflammatory mediators, and b) a cross-talk exists between the innate and the specific immune response, has motivated investigators to take a closer look at innate immunity. To this end, studies have revealed novel inducers, sensors, mediators and effectors in the innate arm of immunity of key relevance to the pathogenesis of lupus. According to the current paradigm, nucleosomes containing nucleic acids (RNA and/or DNA) and other endogenous danger ligands that can bind to pathogen associated molecular pattern receptors are incorporated in apoptotic blebs, which in turn promote the activation of dendritic and B cells and the production of IFN\textsubscript{a} and autoantibodies, respectively. These molecules find their way to specific receptors (toll-like receptors, TLRs; the nucleotide binding and oligomerization domain receptors, NLRs; and the retinoic acid inducible gene-I-like receptors, RLRs) some of which are located intracellularly. Thus in lupus, apoptotic material is not only a source of autoantigens and molecules with adjuvant activity, but also a source of endogenous molecules that can be potent inducers of inflammatory cytokines.

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1. Introduction

Autoimmunity is the reflection of the basic problem confronting living organisms, which is how to defend against foreign invasion, while maintaining control of the defending forces and preserving homeostasis [1]. These mechanisms though are not fail-proof. Dysfunctional innate and/or adaptive immune responses to external pathogens or endogenous molecules derived from a “stressed host” account for an increasingly number of human diseases, both acute and chronic. These mechanisms though are not fail-proof. These mechanisms though are not fail-proof. In 1979, J J Hooks and H M Moutsopoulos published their seminal paper on the detection of immune interferon (IFN) in human SLE [3]. In this paper they reported that type II or immune interferon were present in the serum of lupus patients and showed a good correlation with disease activity. Further characterization of IFN showed that it consisted of IFN-alpha subtypes or a mixture of alpha and gamma IFN. Following a series of publications confirming the study, the finding was largely abandoned and forgotten until Ronnblom and associates showed that apoptotic U937 cells released into lupus patients sera induced normal monocytes to differentiate into DCs. These DCs could capture antigens from dying cells and present them to CD4-positive T cells, raising the possibility that unabated induction of DCs by IFN-alpha may drive the autoimmune response in SLE [5].

Over the ensuing years, demonstration in candidate gene and genome wide association (GWA) studies that several genes encode components of the pathways upstream and downstream of type I IFN-alpha production, further secured the key position of IFN-alpha in the pathogenesis of SLE [6,7]. Importantly, the presence of...
IFN-alpha had also been linked to severity of the disease in microarray studies further supporting the central role of this cytokine in this disease [8–10].

This brief review will highlight selected innate immunity pathways in lupus and the lessons learned.

2. How does lupus start?

Lupus starts with a general autoimmune/preclinical phase characterized by autoantibodies common to other systemic autoimmune diseases, such as anti-Ro and anti-La and proceeds with a more disease-specific autoimmune phase with anti-Sm and anti-RNP antibodies as the disease is becoming clinically apparent [11]. Antibodies against nuclear proteins containing nucleic RNA and/or DNA dominate the immune response in lupus. These observations pose several important questions: What is the source of these autoantigens? How are they recognized? What could account for the dominance of the immune response against nuclear antigens so characteristic of the disease?

During the last years, it has become apparent that increased production of autoantigens during apoptosis, decreased disposal and deregulated handling and presentation, are all important for the initiation of the autoimmune response in lupus (Fig. 1). Nucleosomes containing nucleic acids (RNA and/or DNA) and other endogenous danger ligands that can bind to pathogen associated molecular pattern receptors are incorporated in apoptotic blebs, which in turn promote the activation of dendritic cells (DCs) and B cells and the production of IFN-alpha and autoantibodies, respectively [12–14]. These molecules find their way to specific receptors some of which are located intracellularly (see below). Apoptotic material is not only a source of autoantigens and molecules with adjuvant activity that increases their immunogenicity, but also a source of endogenous molecules that can be potent inducers of inflammatory cytokines following binding to distinct types of innate immunity sensors (see below) [14].

3. Inducers and sensors of innate immunity

In contrast to adaptive immunity which uses specific immune receptors for each antigen, the innate immune system utilizes unique sets of molecules — collectively called pattern recognition receptors (PRRs) — that have been selected to recognize molecular patterns derived from pathogens and damaged cells [1]. These patterns are thought to represent a threat to host’s homeostasis (“danger” signals). As such, PRRs are strategically located on cell membranes, in the cytosolic and in the endosomal compartments of the eukaryotic cells [15].

To date, we recognize at least three distinct types of receptors (Table 1): a) the toll-like receptors (TLRs), which recognize nucleic acids on the cell membranes or on endolysosomal compartments but not in the cytosol; b) the nucleotide binding and oligomerization domain (NOD) receptors (NLRs), which monitor the cytosolic compartment closely interacting with TLR signalling pathways; and c) the retinoid acid inducible gene (RIG)-I-like receptors that recognize RNA or DNA in the cytoplasm (RLRs).

4. Toll-like receptors (TLRs) and autoimmunity in lupus

TLRs are key components of the innate immune system, activating multiple inflammatory pathways and coordinating systemic defence against pathogens. Data from animal models and circumstantial data from humans suggest that inappropriate activation of TLR pathways by endogenous or exogenous ligands may lead to the initiation and/or perpetuation of autoimmune responses and tissue injury [16].

Fig. 1. Sensing of nucleic acids by pattern recognition receptors is a central feature of innate immunity in lupus. This is mediated by Toll-like receptors (TLRs) and cytosolic receptors such as NLRs and RLPs ultimately leading to the production of inflammatory cytokines such as IFNα and IL-1. IFNα promotes the activation/maturation of mDCs that engage quiescent autoreactive T and B cells producing autoantibodies. Once autoantibodies are produced and immune complexes containing ribonucleoproteins and nucleosomes have been formed, their uptake by DCs via the Fc receptors or by B cells via the BCRs facilitates their endosomal delivery to TLRs, resulting in TLR-dependent IFNα production, and promotion of plasma cell differentiation in concert with T cell derived factors, such as IL-21. This amplifies the immune response, which from now on becomes self-sustained.

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In lupus, the best evidence for the involvement of TLRs, exist for TLR-7 and TLR-9. Endosomal TLRs (TLR-3, 7 and 9) are potent activators of DCs and B cells which in turn produce IFN-alpha and autoantibodies respectively (Fig. 1). In mice, production of antinuclear antibodies depends on endosomal toll-like receptors (eTLRs), that bind dsDNA (TLR-9) or ssRNA (TLR7) [17–22]. This may account for the high-frequency and dominance of antinuclear antibody response in lupus [19]. On the other hand, cell surface receptors, such as the B cell receptor (BCR) and FcyRlla, facilitate the endocytosis of nucleic acid containing material or immune complexes sustaining and amplifying the immune response [17,18,21]. Interestingly, chromatin-containing immune complexes can stimulate B cells up to 100-fold more effectively than complexes without nucleic acids, presumably because of combined engagement of BCR and TLR. Dual engagement of the BCR and the TLR can aberrantly activate B cells and breach immune tolerance.

In murine lupus, uptake of immune complexes through a variety of antigen-presenting cells [21,22]. Together, these data suggest that in murine lupus, uptake of immune complexes through a variety of receptors and molecules facilitates their trafficking to endosomal compartment and engagement of TLRs.

In human lupus, we have provided circumstantial evidence implicating the TLR-9 in the pathogenesis of the disease [23]. More specifically, an increased proportion of B cells and monocytes expressed TLR-9 among patients with active SLE compared to patients with inactive disease. TLR activation in combination with T cell derived IL-21 markedly increased B cell differentiation into plasma cells [24]. Patients with lupus nephritis exhibit both glomerular and tubular TLR-9 expression raising the possibility of local activation of renal resident cells by circulating or locally deposited nucleosomes [25].

5. NLRs and lupus

Almost invariably in lupus patients, keratinocytes have increased sensitivity to sun displaying increased rates of apoptosis upon exposure to the UVB component of the solar irradiation [26]. Sun exposure and the resultant UVB irradiation of the keratinocytes in lupus, activates the inflammasome-mediated IL-1b secretion [27] and this may account for the flares following sun exposure in some patients. IL-1 and IL-18 are increased in lupus, while exogenous administration worsens the disease. More importantly, variants in the NALP1 (NLRP1)-inflammasome which activates caspases 1 and caspase 5, have been linked to vitiligo-associated autoimmune diseases including lupus [1,28]. Notably, these caspases convert pyrogenic/immunostimulatory cytokines, such as IL-1 and IL-18 into their active forms. NLR engagement and activation of caspase 1 have been linked to pyropoptotic cell death with disruption of the cell and DNA fragmentation. However, NLR activation can also lead, independently of caspase 1, to pyronecrotic cell death which primarily occurs in autoimmune diseases, without loss of membrane integrity or DNA fragmentation [15,29].

Potential ligands for inflammasomes of relevance to lupus are the self-DNA, which binds to absent-in-melanoma-2 (AIM2) protein (Fig. 1). AIM2 recognizes dsDNA and instead of inducing IFN-alpha, together with ASC (apoptosis-associated speck-like protein containing a CARD), forms an inflammasome that activates caspase 1 

6. RLRs in lupus

In mice, various ligands of NLRs (viral 5’ triphosphate RNA that binds RIG-1 and non-CpG DNA that bind cytosolic DNA sensors) aggravate lupus by enhancing IFN signalling and decreasing regulatory T cells [14]. More importantly, incompletely digested DNA either extracellularly by DNase I or intracellularly by DNase II or III (TREX1) both in mice and humans aggravates the disease by increasing IFN-stimulatory DNA responses [34–36] (Fig. 2). In the case of TREX1, these DNA sequences may be derived from endogenous retroelements and are associated with a prominent vasculopathy also promoted by the concurrent production of IFN-alpha.

7. Type I IFNs: production and effector functions

These comprise a large family of cytokines, including multiple IFN-alpha and the single IFN-beta which are rapidly produced in response to viral infections, and act as critical mediators of host antiviral responses. But how the host detects viral infections and activates IFN production?

Although virtually any cell type can produce type 1 IFNs, the major producers (over 80% of the production) are plasmacytoid dendritic cells (pDCs), a highly specialized DCs subset to sense viral infections [37].

Table 1

| Sensors of the innate immune system with known or postulated relevance in the pathogenesis of lupus. |
|----------------------------------|----------------------------------|-------------------------------|
| **TLRs**                         | **NLRs**                         | **RLRs**                      |
| pDCs, mDCs, MPs, B cells         | DCs, PBMCs, epithelial cells     | mDCs, fibroblasts             |
| Membrane, Cytoplasm              | Cytoplasm                        | Cytoplasm                     |
| TLR2,4,6; DAMPS-HSP, fibrinogen, HMGB1 | NALP1: unknown                  | RIG-1, MDA5: RNA              |
| TLR3,7,9; RNA, DNA               | AIM2: DNA                        | DA1: DNA                     |
| MyD88, TIRAP, TRIF, TRAM         | ASC                              | IPS-1                         |
| NFKb, AP-1, IRF inflammatory cytokines (IFN-α/β, TNF, pro-IL1β) | NFKb (inflammatory cytokines) | NFKb, AP-1, IRF inflammatory cytokines (IFN-α/β, TNF, pro-IL1β) |


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compartments, but this may occur once it is bound to immune independently of TLR activation [37].

A putative intracellular DNA receptor that also produces IFN-alpha is DNA-dependent activator of IFN-regulatory factors (DAI) and MDA5, acting as RNA sensors to mediate TLR-independent IFN-

sensitivity to type I IFN receptor signalling. (Modi

function of STAT4 (signal transducer and activator of transcription 4) increases in macytoid DCs and the IFN responsive gene expression and increase antigen presen-

tation/progression of the disease (initiation), when immune complexes have not been formed, antimicrobial peptides, such as the peptide LL37, bind human-DNA to form complex aggregated structures [38–40]. These are then internalized and retained in early endosomal compart-
ments of pDCs to trigger TLR-9 activation and type I IFN release. Epidermal damage, as in the context of photosensitivity in lupus, may increase the expression of LL37. As a result, human-DNA may continuously trigger TLR-9 mediated IFN responses in pDCs, leading to autoimmune inflammation. Neutrophil extracellular traps (NETs), formed during activation of neutrophils in active lupus, may also protect extracellular DNA from degradation and thus facilitate its entry into the endosomal compartments. NETs represent DNA-containing chromatin structures consisting of a chromatin–DNA backbone with attached antimicrobial peptides and enzymes that trap and kill microbes. Circulating NETs, produced by activated neutrophils, could contain putative autoantigens – as it has been shown in ANCA vasculitides- and stimulate pDCs [41].

In SLE, type I IFNs promote autoimmunity through activation of dendritic cells, T cells, and autoreactive B cells. Induction of IFN-

alpha by administration of an adenovirus encoding IFN-alpha (AdIFN-alpha), or the synthetic dsRNA mimic polyinosinic:polycytidylic acid [poly(I:C)] accelerates the development of autoim-

munity and disease in several strains of lupus-prone mice, including NZB/W mice. Interestingly, augmenting or negating IFN-I activity in murine lupus not only modulates systemic autoimmu-

ity, but also impacts lupus nephritis, suggesting that IFN-I may be acting at the level of the end-organ. Resident renal cells are a dominant source of IFN-I in an experimental model of autoanti-

body-induced nephritis [42] while other investigators have reported severe glomerular proliferative lesions and de novo crescent formation linked to the alternative activation (i.e., IFN-gamma-independent production) of macrophages by IFN-alpha [43]. Together, these findings suggest a role for type I IFNs and alterna-

tively activated macrophages in aggressively proliferative lesions of lupus nephritis and explain the reported association of the IFN-

alpha signature with severe lupus nephritis.

8. Innate immunity pathways are involved both in the initiation and the amplification/progression of the disease

According to the current paradigm [14], the initial event in lupus is thought to be the production of type I interferons by activated pDCs that may or may not involve TLR activation. Once produced, IFN-alpha promotes the activation/maturation of mDCs that engage quiescent autoreactive T and B cells producing autoantibodies. Induction of IFN-

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9. Clinical implications

Well known indicators of lupus flares, such as stress, UV irradiation, and infections—especially viral infections—, through the activation of various sensors of the innate immune response could contribute to initiation or flaring of the disease. Epstein–Barr virus, a major risk factor for lupus, promotes IFN-alpha production by pDCs suggesting that elevated IFN-alpha levels in lupus may be—at least in part—due to aberrantly controlled chronic viral infection [44]. Recently, an acquired defect in TLR signalling has been reported to correlate with remission of SLE in a single patient [45]. Various oligonucleotide TLR antagonists or inhibitors of downstream signalling pathways, with the most notable being the antimalarials, which inhibit TLR and possibly NLR signalling are in use or under development in lupus [46,47]. Antibodies to IFN-alpha are only in clinical trials with early indications of blocking the IFN-inducible gene expression [12]. Inhibition of IL-1 may also be of potential use for some patients with lupus.

10. Lessons learned: «Know thy-self and do not harm»
(“Γνωθί σεαυτόν καὶ μη βάλτε τίνα”)

Nucleic acids, be that either RNA or DNA, are components of every living cells and microbes. Nucleic acids are released in the human body either as a result of physiologic processes, such as apoptotic cell death or as a result of tissue injury and toxic cell death. Thus it is not surprising that organisms have evolved mechanisms to detect aberrant self- or foreign DNA and trigger an innate immune response.

While the immune system needs to have sensors to monitor their release and act accordingly to maintain homeostasis, indiscriminate recognition of endogenous nucleic acids could cause harm. To this end, a number of preventive mechanisms have evolved (Table 2). Lupus clearly illustrates what happens when these mechanisms fail.

While immunologists traditionally sought first sensors for external nucleic acids, research in lupus has provided the impetus to also “look at ourselves” and recognize that sensing of endogenous nucleic acids makes physiologic sense. Once more lupus turned out to be an ideal model system to understand the immune responses to microbial and self-antigens.

11. Advances require astute clinicians and prepared scientists

The observation that in spite of heavy immunosuppression lupus patients are less likely to develop Herpes zoster infections [48], was one of the first leads towards recognizing the antiviral response in SLE and the production of IFN-alpha. Additional observations by astute clinicians have led to better understanding how the immune system «sees itself», with major implications for a variety or chronic diseases including obesity, diabetes, atherosclerosis and Alzheimer’s disease. In these diseases, a variety of stressors stimulate innate immune responses resulting in the production of inflammatory cytokines that mediate several aspects of the diseases. Together with this, has come in recent years the realization that metabolism and inflammation have well preserved, shared pathways that help the organism maintain homeostasis against internal or external perpetrators. Concepts, such as autoinflammation [49], metabolic inflammation and para-inflammation [2] are just the beginning of a new era in nosology, whereby definition and classification of many chronic diseases is based upon common pathophysiological links, rather than on empiricism and potentially misleading disease phenotypes.

We are pleased to participate in this special issue honoring Professor Moutsopoulos. This is part of the series in both the Journal of Autoimmunity and Autoimmunity Reviews honoring contributions ofautoimmunologists [50–55]. Throughout his career H M Moutsopoulos, a physician-scientist in the long tradition of the Arthritis and Rheumatism Branch of NIH, skillfully and consistently served both, the “physician” and the “scientist” components in a balanced way. Physician scientists, an endangered species in our days, need to be better nurtured and supported if we are to expect major advances in the understanding and treatment of lupus.

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