

Identification of type I interferon-associated inflammation in the pathogenesis of cutaneous lupus erythematosus opens up options for novel therapeutic approaches

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Abstract: Cutaneous lupus erythematosus (CLE) is one of the most common dermatological autoimmune disorders worldwide. Recently, several studies provided evidence for a pathogenic role of type I interferons (IFNs) in this disease. Plasmacytoid dendritic cells are major type I IFN producers in CLE skin lesions. Type I IFNs are able to induce the expression of several proinflammatory chemokines, including CXCL9 and 10, and enhance the cytotoxic capacity of infiltrating cells. Additionally, adhesion molecules and chemokine receptors, such as intercellular adhesion molecule-1,

cutaneous lymphocyte antigen, E-selectin, CCR4 and CXCR3, are involved in the recruitment of potentially autoreactive lymphocytes into the skin. Here, we review the role of type I IFNs, adhesion molecules and chemokine receptors in CLE and discuss options for novel therapeutic approaches.

Key words: CCR4 – chloroquine – cutaneous lymphocyte antigen – CXCR3 – interferon- α – methotrexate – oligodeoxynucleotides – skin

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Clinical findings in cutaneous lupus erythematosus

Lupus erythematosus (LE) is a complex autoimmune disease with a broad spectrum of clinical manifestations reaching from localized skin lesions to systemic disease. Classically, pure cutaneous subtypes [cutaneous LE (CLE)] are distinguished from systemic LE (SLE). In CLE the disease is limited to the skin, in SLE it presents with involvement of internal organs such as nephritis, pleuritis, pericarditis, arthritis and cerebral vasculitis. Overlaps and transitions from CLE into SLE are observed in 10–40% of patients (1). Chronic discoid LE (CDLE) is the most common subtype of CLE, which presents with scarring erythematous macules and plaques, localized to the face or to the capillitium (localized CDLE). The same lesions may rarely also appear in a disseminated distribution pattern (disseminated CDLE), additionally involving neck, shoulders, arms, trunk and legs. Subacute CLE (SCLE) represents 10–15% of CLE cases (2). It appears with annular or gyrated macules and plaques in sun-exposed areas, including shoulders, back, arms but only infrequently involves the face (2).

Rare subtypes of CLE include lupus profundus (LEP), characterized by panniculitis and chilblain LE, which presents with pernio-like skin lesions (3). Urticarial lesions appearing in sun-exposed areas are typical for lupus tumidus (LET) (4). Typical findings of different CLE subsets are given in Fig. 1.

Histological picture

A lymphocytic interface dermatitis with hydropic degeneration of the basal epidermal layer accompanied by a dense perivascular and periadnexal inflammatory infiltrate is the main histological characteristic of CLE. In general, CDLE presents with a more extensive inflammatory picture than SCLE (1). LET is dominated by a perivascular lymphocytic infiltration, LEP by a lobular panniculitis. The inflammatory infiltrate in CLE is mainly composed of lymphocytes and histiocytes (5).

Autoantibodies in CLE

Antinuclear antibodies are found in 20–80% of cases, depending on the underlying subtype (1,6). Anti-SSA/Ro and anti-SSB/La autoantibodies are frequently found in



Figure 1. Clinical and histological findings in CLE compared with the expression pattern of the type I IFN marker MxA. The figure depicts typical examples of some CLE subtypes: chronic discoid LE (CDLE) presents with scarring erythematous macules and plaques. Histologically, a junctional interface dermatitis as well as dense perivascular and perifollicular lymphocytic infiltrates are typically seen. Subacute cutaneous LE (SCLE) appears with annular or gyrated plaques and macules in sun-exposed areas. Characteristic histological findings include interface dermatitis with atrophy and basal hydropic degeneration of the epidermis. LET shows urtica-like plaques without scaling. Accordingly, characteristic histological findings include dense perivascular lymphocytic infiltration, but no epidermal involvement. LEP affects mainly the subcutaneous tissue. A mostly lobular panniculitis is typically found in lesional histology. Importantly, lesional expression of the antiviral protein MxA, which is a specific marker for type I IFN signalling, perfectly reflects the inflammatory pattern typically seen in the different CLE subsets. In CDLE, strong MxA expression is seen not only in the epidermis, but also in perivascular and perifollicular areas, while SCLE presents with a mainly epidermal MxA expression pattern. LET shows an accentuated perivascular MxA expression, LEP presents with MxA production within the subcutaneous tissue.

CLE patients and associate closely with photosensitivity (1,7). Other antinuclear antibodies such as anti-dsDNA, anti-Sm or anti-rRNPs which are associated with organ involvement (lupus nephritis, central nervous system involvement, serositis) are only infrequently detected and may indicate a more severe course (8).

The pathogenic relevance of autoantibodies in LE is still intensively discussed. Maternal transition of SSA/Ro immunoglobulin G antibodies during pregnancy, inducing neonatal LE in an otherwise healthy child with a clinical picture resembling SCLE and congenital heartblock, supports the pathogenic relevance of these antibodies (9). Nuclear antigens that are expressed on the surface of apoptotic keratinocytes after extensive UV provocation and bind circulating anti-SSA/Ro and SSB/La antibodies may be involved in exacerbation of SCLE and SLE after sunlight

provocation (10–12). However, several patients with circulating SSA/SSB autoantibodies in their serum never develop skin lesions (13).

Pathogenesis

Lymphocyte recruitment in CLE

Molecular mechanisms orchestrating lymphocyte recruitment into inflammatory skin lesions were first identified in atopic dermatitis and psoriasis (14). Similar observations were since been reported for various types of skin inflammation, including CLE. Several epidermal surface and adhesion molecules are upregulated in CLE skin lesions (5,15,16). For example, strong intercellular adhesion molecule (ICAM)-1 expression in lesional keratinocytes, dermal inflammatory cells and endothelial cells was found in one study investigating the expression of this adhesion molecule in 18 CLE patients by immunohistochemistry (5). LFA-1 was confined to the dermis, suggesting that attachment between keratinocytes or endothelial cells and activated T lymphocytes via ICAM-1/LFA-1 may be a possible mechanism of target/effector recognition in CLE (5). Additionally, expression of endothelial adhesion molecules VCAM-1, E-selectin and P-selectin is upregulated (17), while enhanced soluble E-selectin in CLE serum correlates with disease activity (18). Recent studies also revealed the expression of cathepsin L in CLE skin lesions (19). We recently described the expression of CCR4+ on CD4+ and CD8+ T lymphocytes in CLE skin lesions. The respective ligand TARC/CCL17 was strongly expressed in lesional skin. Patients with active CLE had significantly upregulated TARC/CCL17 serum levels (20). High numbers of circulating cutaneous lymphocyte antigen (CLA)+ CCR4+ CD8+ cells were found in patients with disseminated scarring CDLE (20). These peripheral CLA+ T cells showed an enhanced expression of cell activation markers (21), and might reflect the effector population of potentially autoreactive cytotoxic T cells typically seen within scarring CDLE skin lesions (22).

Role of type I interferon in SLE

Our observations raised the question, which proinflammatory factors might be responsible for the inflammatory response leading to lymphocyte skin recruitment in CLE. Here, earlier observations in SLE pointed to a central pathogenic role of type I interferons (IFNs).

Clinical observations suggested a pathogenic role of type I IFN in LE for more than 20 years. Patients with acute SLE often present with flu-like symptoms such as fever, fatigue, rash, arthralgia and myalgia. These symptoms are now thought to reflect high serum type I IFN levels which correlate with both disease activity and severity (23,24). Also, exacerbation of SLE after IFN- α treatment has been

described (25). More recent studies examining gene expression in SLE have identified a type I IFN signature in patients with active disease (26,27).

Interferons were first described in 1954 by Lindemann, who observed that culture supernatants of virus-infected cells include a soluble factor that protect from further viral infection (28). The type I IFNs are a large group of cytokines that have one common receptor, called IFN- α/β R (29). They induce the expression of a number of genes that inhibit virus replication and support the immune system (30), including the MxA protein which is a cytoplasmatic GTPase with activity against RNA viruses (31).

Polymorphisms of IFN-related genes were recently found to be associated with an increased susceptibility for the development of SLE. Ronnblom and co-workers analysed single-nucleotide polymorphisms (SNP) in 11 type I IFN-related genes in SLE patients. SNPs in two genes, Tyk-2 and interferon regulatory factor (IRF)-5, were closely associated with SLE (32). Tyk-2 binds to IFNAR-1 and plays an important functional role for signalling via the IFN- α/β R (33). IRF-5 is central for the induction of type I IFN production by viruses and TLR-7/8 agonists. It is constitutively expressed in plasmacytoid dendritic cells (pDCs) and increases the expression of several genes involved in cell signalling, apoptosis and immune activation (33,34).

A pathogenic role of type I IFN in SLE is also supported by several experimental mouse models. Adam et al. found that in autoimmune NZB/W mice injected with IFN growth was delayed, survival was decreased, and the severity of the glomerulonephritis was enhanced. Additionally, IFN increased the titre of serum anti-ssDNA and anti-soluble nucleoprotein antibodies (35). Autoimmune lupus NZB \times NZWF1 (B/WF1) mice, treated with IFN-releasing agents, develop a more severe disease including glomerulonephritis and increased titre of antinuclear antibodies (36). Additionally, type I IFNs control the onset and severity of autoimmune manifestations in *lpr* mice, while introducing a null mutation for the IFN-receptor gene into the *lpr* background clearly reduces SLE-like disease (37,38).

Cellular and molecular mechanisms of type I IFN production

Type I IFNs play a central role in viral immune defence and all cells carry specialized receptors which recognize viral nucleic acids and induce IFN production. The structure of type I IFNs is highly conserved, ranging from 70–98% amino acid identity between the 13 different IFN- α subtypes to 35% identity between IFN- α and IFN- β , underlying the importance of this system during evolution (39). Type I IFNs can be produced by every mammalian cell (30). However, among the blood cells a small population, representing only 0.1% of PBMCs, is able to produce large amounts (1000-fold more than other cells) after viral infec-

tion (40). In 1983, Ronnblom and co-workers were the first who described this cell type which strongly produced IFN- α when co-cultured with WISH human amnion cells or K562 tumour cells and named these cells 'natural IFN-producing cells' (NIPC) (41). Today it is clear that these NIPCs, which are now called pDCs, are key cells in linking the innate and adaptive immune response (33,42,43).

Viruses are typical type I IFN inducers, but some bacteria and protozoans can also promote IFN production (44). Molecular mechanisms of IFN induction depend on the activation of specific receptors, so called pattern-recognition receptors, that recognize 'pathogen-associated molecular patterns' (45). The family of 'Toll-like receptors' (TLR) represents the best investigated group among these. Microbial TLR ligands include a wide range of pathogen-associated molecules such as lipopolysaccharides, lipopeptides, viral RNA and bacterial DNA (46–52). TLR7 was originally identified as a receptor for viral ssRNA, TLR9 recognizes bacterial or viral DNA on the basis of a high frequency of hypomethylated CpG-motives in non-mammalian DNA (52,53). Most TLRs use MyD88 for intracellular signal transduction. Others, such as TLR3 utilize specifically TRIF (Toll/IL-1 receptor domain-containing adaptor inducing IFN- β) as adaptor. As shown in Fig. 2, further adaptor molecules including the IRF-3, 5 and 7 are needed to induce finally the expression of type I IFNs and other proinflammatory proteins (33). Very recently, additional TLR-independent ways of IFN induction have been identified. The cytoplasmatic helicases Mda5 and RIG-I also recognize virus-associated molecular patterns (Mda5: polyIC; RIG-I: 5'-Triphosphate RNA) and induce IFN expression via IFN-beta promoter stimulator 1 (IPS-1, see Fig. 2) (54–56).

Mechanisms of IFN production in SLE

In 1999, Vallin et al. observed that DNA-containing immune complexes (ICs) of SLE patients induce IFN- α production by pDCs (57). The occurrence of these 'interferonic' immune complexes is associated with active disease (58).

Today it is known that these 'interferogenic' ICs may contain endogenous nuclear antigens and anti-dsDNA or anti-U1snRNP autoantibodies, respectively (59,60). The mammalian DNAs and RNAs are potent self-antigens for TLR7 and TLR9 (61), which are constitutively expressed in the endosomes of pDCs (62). The stimulatory effect of these ICs is dependent on the presence of the Fc γ RIIa (CD32) receptor on the cell surface (63). DNA-containing ICs of SLE patients have been shown to stimulate pDCs to produce cytokines and chemokines via a cooperative interaction between TLR9 and CD32. These ICs transiently co-localize to a subcellular compartment containing CD32 and TLR9, and CD32+, but not CD32-, pDCs internalize

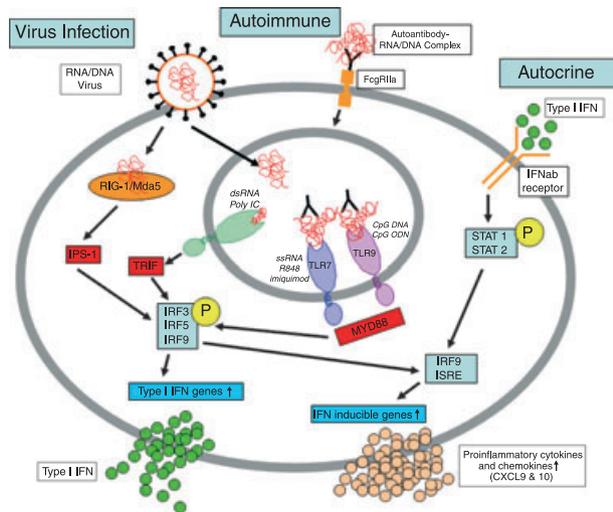


Figure 2. Mechanisms of type I IFN induction in plasmacytoid DCs. From an evolutionary point of view the type I IFN system primarily developed for antiviral host defence. Foreign RNA and DNA fragments are recognized by intracellular receptors. In endosomes, the TLRs 3, 7 and 9 play a major role by recognizing nucleic acid fragments and inducing the transcription of type I IFN genes via MyD88 and TRIF signalling pathways, respectively. Additional cytoplasmatic sensors for viral nucleic acids are the helicases RIG-I and Mda5, which induce type I IFN expression via IPS-1. This system might be dysregulated under autoimmune conditions such as SLE. Here, complexes of autoantibodies (anti-dsDNA/anti-U1snRNP) and nuclear fragments bind to Fc γ RIIIa receptors on the cell surface. After endocytosis, the RNA/DNA fragments are able to induce TLR-dependent IFN expression. Additionally, a self-amplifying autocrine loop is known which perpetuates the expression of IFN inducible genes via IFN- $\alpha\beta$ receptor and phosphorylation of STAT 1 and 2. Model modified after Ronnblom et al. (33).

and respond to SLE-ICs (64). TLR9 controls anti-DNA autoantibody production in murine lupus (65). Engagement of the TLR9 by various ligands, including CpG-motifs, can cause or aggravate pathogenic autoantibody production and cytokine secretion in lupus (66). The U1snRNA component of U1snRNP immune complexes, found in patients with SLE, acts as an endogenous 'self' ligand for TLR7 and triggers IFN- α production in pDCs (45). RNA-containing ICs stimulate pDCs from wild-type (but not TLR7-deficient) mice to produce IFN- α (45). Pretreatment of pDCs with anti-CD32 antibodies blocks IFN induction in pDCs (63). Furthermore, Rutz et al. demonstrated that chloroquine directly blocks TLR9-CpG-DNA interaction and acts as a TLR9 antagonist, probably due to the inhibition of endosomal acidification (67). A concise model for IFN induction in pDCs is included in Fig. 2.

The source of DNA and RNA fragments in SLE patients is not yet identified, but recent studies showed that apoptotic or necrotic cells can generate interferonic DNA/RNA material (59,68). As SLE patients have a reduced clearance of dying cells, apoptotic RNA and DNA fragments are

surely available in SLE patients in vivo (69,70). Anti-dsDNA and anti-U1snRNP autoantibodies are only seen in a minority of CLE patients (6), making this concept not directly transferable to CLE. However, apoptotic malfunction holds also true for CLE. Kuhn and co-workers found that apoptotic cells accumulate in the skin of patients with CLE after UV irradiation, probably as a result of impaired or delayed clearance. They hypothesized that the non-engulfed cells may undergo secondary necrosis and release proinflammatory compounds and potential autoantigens, which may support formation of skin lesions in this disease (71).

Type I IFNs and associated chemokines in CLE

Type I IFNs also play an important pro-inflammatory role in the pathogenesis of CLE. In 1995, Fah et al. found strong expression of the antiviral-MxA protein, which is a specific marker for type I IFN production, in CLE skin lesions. These findings implicated viral involvement or activation of the IFN system by thus far unknown mechanisms in this disorder (72). Some years later, Blomberg et al. (73) and Farkas et al. (74) identified large numbers of IFN- α expressing pDCs in CLE skin lesions. Our own observations showed that lesional type I IFN signalling is closely associated with the extent of the inflammatory infiltrate in several CLE subsets, suggesting a direct proinflammatory role of type I IFN in this condition (22,75,76). Accordingly, CLE patients with widespread skin lesions often present with typical type I IFN-mediated symptoms such as fever and fatigue (77). We found a strong intracellular MxA expression in circulating blood lymphocytes, which was closely associated with the extent of skin lesions in our investigated CLE patients (75). These results were recently confirmed by gene expression analyses of lesional CLE skin biopsies, which revealed a strong type I IFN signature including expression of the IFN- α -inducible genes IRF7 and MxA (78).

In subsequent studies we investigated the type I IFN-driven inflammation in CLE. We showed that local production of type I IFNs in CLE induces Th1-biased inflammation via induction of IFN-inducible chemokines such as CXCL10 leading to the recruitment of chemokine receptor CXCR3 expressing T cells into skin lesions (75). IFN- α was able to induce IP10/CXCL10 expression in keratinocyte culture. In lesional skin, CXCL10 was expressed in basal epidermal areas with the strongest junctional inflammation (75). CXCR3+ T cells were found in large numbers in lesional skin, while the amount of circulating CXCR3+ lymphocytes was decreased, suggesting recruitment of these cells into the skin (75). Interestingly, CXCR3+ cytotoxic lymphocytes dominated the inflammatory infiltrate in scarring CDLE, supporting a role of CXCR3+ cytotoxic effector cells in this disorder (22). Our data of enhanced lesional CXCL10 expression were confirmed by Meller et al. who additionally found a strong mRNA expression of CXCL9,

another CXCR3 ligand (78). Based on these findings we developed a pathogenic model that explains the inflammation that is characteristic for CLE lesions.

Pathogenic model of chronic inflammation in CLE

Lesional induction of type I IFN expression appears to be the hallmark of skin inflammation in CLE. Several earlier studies dealing with the pathogenesis of CLE revealed a strong induction of type I IFNs and type I IFN-inducible chemokines, respectively (73–75,78). However, the primary stimulus that induces IFN expression is not yet clear. Clinical observations implicate that several different factors such as UV-light, infection, trauma, drugs and TLR activation play a role. Impaired necrosis/apoptosis in the skin appears to be a common mechanism of these different trigger factors for lesional inflammation. The release of nuclear fragments could stimulate (on the background of impaired clearance by phagocytes) lesional IFN production (59,71,79). Type I IFNs are known to enhance the cytotoxic capacity of circulating lymphocytes (80), and induce the production of antiviral proteins (MxA, IFI27) as well as the chemokine CXCL9 and 10, which are ligands for the chemokine receptor CXCR3 (75). In turn, these chemokines cause the recruitment of autoimmune CXCR3+ lymphocytes and pDCs into the skin (81,82). At least three different self-perpetuating mechanisms should be mentioned, which might amplify this inflammation: First, CXCR3+ cytotoxic lymphocytes have been shown to carry CXCL10 in their granules, which is poured out in the target tissue and might provide direct 'lymphocyte self-recruitment'. These mechanisms were shown in lichen planus and lichen sclerosus et atrophicus, autoimmune skin disorders that share several common features with CLE, such as interface dermatitis (83,84). Second, immigrated pDCs are able to produce large amounts of type I IFNs, which again perpetuates the lesional inflammation (73,85). Third, lesional inflammation leads to cell destruction and impaired apoptosis, which in turn induces the expression of several proinflammatory mediators. The model is depicted in Fig. 3.

Therapeutic options in CLE

Treatment of CLE patients is based on three columns: prevention, topical treatment and systemic therapy. As most CLE patients are photosensitive, prevention includes avoidance of UV-light exposure, wearing of sun-protective clothing and continuous use of sunscreen. Topical corticosteroids represent the most frequently used topical drug-family, but recent studies highlighted also the good efficacy of topical calcineurin inhibitors (tacrolimus/pimecrolimus) in CLE (86,87). Systemic corticosteroids still represent the first-line treatment for systemic therapy. However, due to the well known side effects, their use is limited in

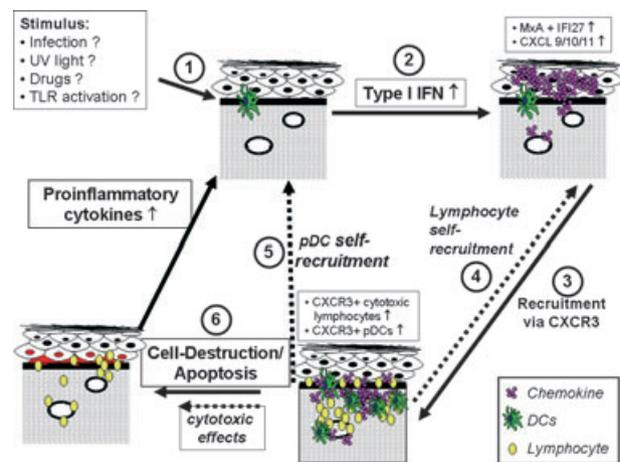


Figure 3. Hypothetical model of type I IFN-associated lymphocyte recruitment in CLE. A primary (yet unknown) stimulus (1) induces the lesional expression of type I IFNs in the skin (2). Type I IFNs are known to induce the production of antiviral proteins (MxA, IFI27) and the chemokine CXCL9 and 10, which are ligands for the chemokine receptor CXCR3. Following, CXCR3+ lymphocytes and pDCs are recruited into the skin (3). A least three different self-perpetuating mechanisms should be mentioned, which might be responsible for the chronic inflammation seen in CLE: First, CXCR3+ cytotoxic lymphocytes have been shown to carry CXCL10 in their granules (4), which is spilled out in the target tissue and might provide direct 'lymphocyte self-recruitment'. Second, immigrated pDCs are able to produce large amounts of type I IFNs, what again perpetuates the lesional inflammation (5). Third, lesional inflammation leads to cell destruction and impaired apoptosis (6), which again induces the expression of several proinflammatory mediators.

multimorbid patients and for long time application (77). Therefore, anti-malarials, especially chloroquine and hydroxychloroquine, are the first choice in long-time treatment (88). In recalcitrant patients, resistant to topical treatment and anti-malarials, several immunomodulatory and immunosuppressive drugs are used (77). Here, we made the best experience with low-dose methotrexate (89–91).

Chloroquine/hydroxychloroquine

Anti-malarials are established drugs in the treatment of CLE and SLE for decades (88,92). In 1894, Payne was the first who used quinine, an alkaloid derived from the bark of the cinchona tree, in order to manage CLE lesions. During the Second World War, at least three million American soldiers took mepacrine, a synthetic alkaloid, for malaria prophylaxis. Among those, an improvement of preexisting LE and rheumatoid arthritis was observed (93). Due to side effects like aplastic anaemia, further anti-malarial drugs were developed during the following years including chloroquine, hydroxychloroquine and quinacrine (92). Among those, chloroquine and hydroxychloroquine are today the most frequently used agents (88). Several anti-inflammatory and immunosuppressive mechanisms have been discussed

to be important for the agents' mode of action (88), including lysosomal stabilization (94), suppression of antigen presentation (95), and the inhibition of proinflammatory prostaglandins and cytokines, e.g. IL-1 and IL-6 (96,97). Chloroquine is a weak diprotic base that can pass through the lipid cell membrane and preferentially concentrate in acidic cytoplasmic vesicles. The resulting slight elevation of pH within these vesicles is supposed to influence the immune response (98). Now, recent studies indicate that the fact that chloroquine blocks pH-dependent formation of the endosomal TLRs 7 and 9 might be a central key to understand the therapeutic function of this drug (66,67). Chloroquine inhibits the activation of pDCs by SLE-immune complexes *in vitro* (60).

The hypothesis that chloroquine functions mainly by blocking pH-dependent maturation of endosomal TLRs is supported by the following clinical observations: (i) the drug needs several weeks for a therapeutic effect and (ii) chloroquine acts mainly as protective agent that protects from new exacerbation but not due to direct anti-inflammatory properties (77).

Methotrexate

Methotrexate is a folic acid antagonist, developed in the first half of the last century for the treatment of leukaemia. It irreversibly blocks nucleic acid synthesis by inhibiting purine synthesis. In higher doses, this results in reduced production of immune cells and direct immunosuppression, partially due to enhanced lymphocyte apoptosis (99,100). Low-dose MTX is an effective therapeutic agent in several autoimmune conditions, including SLE and CLE (89,101). MTX was designed as folic acid antagonist, but its effectiveness in autoimmune disorders is independent from this properties and it is still effective, if folic acid is substituted parallelly (102). The agent has several anti-inflammatory properties. MTX promotes adenosine release and inhibits transmethylation reactions (100). It inhibits neutrophil immigration into tissue, the LTB₄ synthesis in neutrophils, the synthesis of interleukin 1 and is able to suppress cellular immunity (100).

Recent studies highlighted anti-migratory effects of MTX in the treatment of skin diseases. Sigmundsdottir et al. found that MTX causes a dose-dependent suppression of T-cell activation and adhesion molecule expression, and this was not due to lymphocyte apoptosis (103). MTX reduces the capability of circulation lymphocytes to migrate into the skin by suppressing the ICAM-1, decreasing the expression of the CLA on mononuclear cells in the blood and a downregulation of endothelial E-selectin. This results into an approximately threefold decrease of mononuclear leucocyte infiltration of lesional skin (103,104). The thesis that MTX in CLE acts mainly by blocking of cell migration and not by direct immunosuppression is supported by our own observations: MTX treatment enhances the number of

circulating lymphocytes in CLE patients with lymphocytopenia, probably reflecting an impaired lymphocyte recruitment under therapy (89).

Novel molecular targets for therapy

Chemokines and adhesion molecules as targets in CLE treatment

Raising insights into mechanisms of lymphocyte skin recruitment and insights in the proinflammatory role of type I IFN-driven skin-inflammation have opened the door for new generations of drugs in dermatological treatment. Future therapeutic strategies might focus in part on blocking the skin migration of potentially autoreactive lymphocytes. Efalizumab, a humanized murine antibody directed against CD11a that blocks the LFA-1↔ICAM-1-dependent recruitment of lymphocytes into the skin, has been shown in single cases to improve CLE skin lesions and dermatomyositis, a disease which shares several pathogenic properties with CLE (105,106).

Furthermore, monoclonal antibodies that target chemokine receptors such as CCR4 and CXCR3 might also block the recruitment of autoimmune T cells into the skin. CCR4 is broadly expressed on activated T cells and modulates T-cell migration into the inflamed skin. It is best known as a drug target for airway inflammation and atopic dermatitis (107). Selective small molecule antagonist 'Compound 8c' of CCR4 has been shown to be effective in a murine allergic inflammation model (108). As CCR4 is involved in lymphocyte migration in CLE, it represents also a potential target in this disorder (20). CXCR3 represents an additional potential drug target. Today, there is no clinical experience with anti-CXCR3 antibodies or blocking of the respective ligands (CXCL-9, 10 and 11) in this disorder. However, blocking the CXCR3–CXCL10 axis is under consideration in other chronic inflammatory disorders such as psoriasis, inflammatory bowel disease, chronic obstructive lung disorder and allergic diseases (109–112).

Anti-interferon treatment in CLE

In addition to anti-inflammatory strategies focussing on adhesion molecules and chemokines to prevent lymphocyte migration into tissue, the type I IFN system on its own provides several potential molecular targets. This anti-IFN treatment might focus on (i) type I IFN induction, (ii) soluble IFN and the IFN- $\alpha\beta$ receptor or (iii) on the intracellular IFN pathway (113).

Animal studies have revealed that TLR9 plays a central role for IFN induction in autoimmune disorders (114). Engagement of the TLR9 by various ligands, including CpG-motifs, can cause or aggravate pathogenic autoantibody production and cytokine secretion (66). Therefore, attempts to neutralize this activity using inhibitory oligo-

nucleotides could be a promising therapeutic option for LE (66). Very recently, INH-ODNs were developed which block all downstream signalling events in TLR9-responsive cells and in part also TLR7 signalling pathways. These ODNs block type I IFN induction by RNA and DNA immune-complexes in human pDCs in vitro (61) and inhibit the development of SLE in animal model (66). Plasmacytoid DCs, which are supposed to be the major source of type I IFNs in CLE skin lesions might be another target to inhibit type I IFN production. These cells specifically express the surface receptor BDCA-2 and -4. Ligand of BDCA2 receptor using a monoclonal antibody was shown to suppress the type I IFN induction in pDCs (115). The addition of anti-BDCA-2/4 antibodies to normal PBMCs reduced the production of type I IFNs induced by viruses and sera from SLE patients up to 80% (113,116).

Direct anti-IFN- α antibodies are additional candidates for new developments in LE treatment (117,118). The strategy to target proinflammatory cytokines directly by monoclonal antibodies has been shown to be sufficient in psoriasis, using anti-TNF- α antibodies (119). A humanized monoclonal antibody was developed to target IFN- α in SLE and other autoimmune disorders, but in vivo data are not yet available (117). As all type I IFNs bind to the same receptor (IFN- α BR), this receptor provides also a potential drug target (113).

Subsequently, targeting the intracellular type I IFN pathway downstream IFN- α BR-activation might be a promising tool in the treatment of CLE. Inhibition of the p38 mitogen-activated protein kinase (p38 MAPK) pathway is effective in lupus animal models (120). p38 MAPK is required for type I IFN-mediated ISGF3 formation (113). Histone deacetylase inhibitors reverse the skewed expression of multiple genes involved in SLE (121). Several compounds targeting the intracellular IFN pathways are currently undergoing clinical studies in autoimmune disorders such as rheumatoid arthritis (113) and also provide potential therapeutic options in CLE.

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