Clinical Immunology 174 (2017) 10–17

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Pso p27, a SERPINB3/B4-derived protein, is most likely a common autoantigen in chronic inflammatory diseases

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ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form 1 November 2016
accepted with revision 13 November 2016
Available online 15 November 2016

Keywords:
Autoimmune diseases
Pso p27
SERPINB3/B4
Mast cells
Psoriasis

ABSTRACT

Autoimmune diseases are characterized by chronic inflammatory reactions localized to an organ or organ-system. They are caused by loss of immunologic tolerance toward self-antigens, causing formation of autoantibodies that mistakenly attack their own body. Psoriasis is a chronic inflammatory autoimmune skin disease in which the underlying molecular mechanisms remain elusive. In this review, we present evidence accumulated through more than three decades that the serpin-derived protein Pso p27 is an autoantigen in psoriasis and probably also in other chronic inflammatory diseases.

Pso p27 is derived from the serpin molecules SERPINB3 and SERPINB4 through non-canonical cleavage by mast cell chymase. In psoriasis, it is exclusively found in skin lesions and not in uninvolved skin. The serpins are cleaved into three fragments that remain associated as a Pso p27 complex with novel immunogenic properties and increased tendency to form large aggregates compared to native SERPINB3/B4. The amount of Pso p27 is directly correlated to disease activity, and through formation of complement activating immune-complexes, Pso p27 contribute to the inflammation in the skin lesions. SERPINB3/B4 are expressed in skin fibroblasts and keratinocytes, but normally absent in mast cells. Overexpression of the serpins may be induced by inflammation and hypoxia, resulting in mast cell uptake *via* yet unknown mechanisms. Here the generation and subsequent release of Pso p27 aggregates may promote an inflammatory loop that contributes to the chronicity of psoriasis and other autoimmune diseases.

1. Introduction

Autoimmune disease is a common term of diseases caused by immune responses against self-antigens. Such responses may be triggered *via* different mechanisms, including abnormal alteration of self-antigens or exposure to microbial antigens harbouring epitopes similar to those found in humans [1]. The disease is defined according to the affected organ or organ system and it has been postulated that the inflammation is caused by immune reaction toward tissue specific autoantigens. However, despite considerable efforts the search for such autoantigens has not given breakthroughs in our understanding of disease mechanisms.

There are some important facts that should be taken into account in the search for autoimmune disease mechanisms: 1) The inflammation is usually restricted to parts of the affected organ or organ system. This argues against a tissue specific autoantigen, which would rather mediate involvement of the whole organ. 2) The inflammation is in principle chronical. This argues for a positive feedback mechanism where inflammatory consequences give rise to more inflammation. 3) The coincidental occurrence of more than one autoimmune condition in the same patient. This indicates common disease mechanisms in various autoimmune diseases.

Psoriasis is an inflammatory skin disease that fulfils all three criteria. Several studies suggest that activated T-cells play a role in triggering and/or maintaining the disease [2]. It is also a clear HLA-association with psoriasis, consistent with T-cell mediated autoimmunity, and the highest relative risk is associated with HLA-C*06:02 [3]. More than 40 years ago Jablonska et al. [4] reported more IgG bound to the epidermis and stratum corneum of psoriatic lesions than in other inflammatory and hyperkeratotic diseases. Since then, several psoriatic autoantigens have been suggested, including keratins structurally mimicking bacterial M-proteins [5], ezrin, maspin, PRDX2 and HSP27 [6] basement membrane laminin [reviewed in [7]], the antimicrobial peptide LL37 [8] and the melanocyte protein ADAMTSL5 [9]. However, neither of these have been found to be present among all psoriasis patients. Conversely, Pso P27, a proteolytically processed form of the SERPINB3 and SERPINB4, is abundantly expressed in psoriatic lesions from all patients we have examined so far while it is not found in unaffected skin in the same patients. The present review will outline research through

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http://dx.doi.org/10.1016/j.clim.2016.11.006

more than three decades leading to the identification of Pso p27, highlighting its potential role as an autoantigen in psoriasis as well as in several other inflammatory diseases.

2. The presence of virus-like particles in psoriasis

In an approach to identify etiological factors in psoriasis, it is appropriate to compare psoriatic skin lesions and uninvolved/healthy skin from the same patient. Several decades ago electron microscopic analyses of psoriatic skin lesions demonstrated subcellular virus-like particles that were not present in the uninvolved healthy skin ([10,11], Fig. 1). Membrane coated virus-like particles were released to the culture medium during cultivation of tissue from psoriatic lesions [11] and similar particles were obtained from the urine of psoriatic patients with extensive disease activity [12,13]. Purification of the particles by isopycnic ultracentrifugation and subsequent denaturing size-exclusion chromatography demonstrated that the particles contained three core proteins with estimated molecular masses of 12, 15 and 27 kD, respectively, [13]. Based on their morphology, buoyant density and molecular mass of the core proteins, it was hypothesised that the particles could be endogenous retroviruses. This was also in line with a previous observation of retroviral immune complex deposits in skin biopsies in another autoimmune disease, systemic lupus erythematosus [14]. However, neither polyadenylated RNA nor reverse transcriptase could be detected in the particles [15]. Even if the particles did not turn out as infectious agents, their potential role in the pathogenesis of psoriasis remained unaddressed.

3. Immune reaction against protein constituents of virus-like particles — the autoantigen Pso p27

Chronic inflammation with infiltration of inflammatory cells in the skin lesions is a characteristic feature in psoriasis. However, despite extensive research, no infectious candidate has been identified as causal agent in the immune reactions. As the virus-like particles were present in the skin lesions only, it seemed appropriate to investigate whether the particle proteins could be immunogenic and play a role in the immune reactions in psoriasis. To follow this assumption the major internal protein from the virus-particles, p27, was selected as reference protein. Using rabbit antiserum against p27 a cross-reacting protein was extracted from psoriatic scale. Even if the particle-associated protein and the serological cross-reacting protein obtained from psoriatic lesions had similar molecular mass they were not necessarily identical. Thus, to differentiate the two proteins, the latter was named Pso p27 [16].

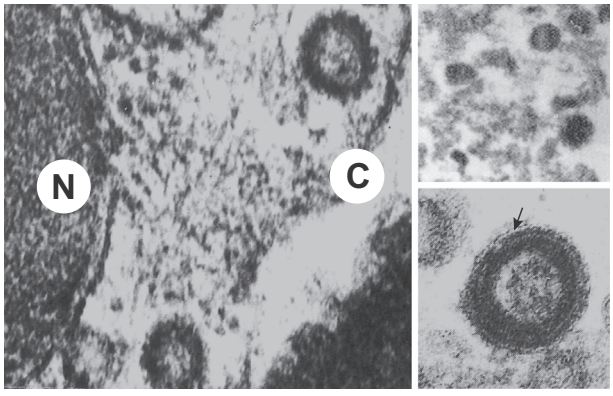

Fig. 1. Intracellular spherical bodies in psoriatic lesion and extracellular membrane coated particles likely released *via* budding or cell destruction demonstrated by electron microscopy [11].

Large amounts of Pso p27 can be extracted from psoriatic scale and with reference to serum albumin the concentration of Pso p27 in scale exceeds that in serum with a factor of 10^6 (17). This underlined the potential of Pso p27 as a disease associated protein. To elucidate the potential role of Pso p27 as an autoantigen, antibodies in psoriatic scale extract were analysed by indirect ELISA with p27 or Pso p27 as antigen [18]. The relative concentration of anti-Pso p27 specific antibodies extractable from psoriatic scale exceeded that of serum from the same patient with a factor of 10^3 (Fig. 2). These observations indicated the role of Pso p27 as a locally generated immunogen [18,19]. Furthermore, immune-complexes formed by the Pso p27 antigen and corresponding antibodies from psoriatic scale were shown to activate the complement system and thus emphasized the significance of Pso p27 in the inflammatory process [20]. When scale antibodies were used in an approach to identify candidate antigens in the psoriatic scale extract, the Pso p27 antigen was identified as the most prominent candidate [19]. Furthermore, use of scale antibodies in indirect immunofluorescence microscopy of thin sections of psoriatic lesions revealed antigens in a sub-fraction of dermal cells identical to those recognised by anti-Pso p27 antibodies [Fig. 3, [19]].

Based on these findings it was concluded that Pso p27 appear as a major antigen in the immune reactions in psoriasis [19].

4. Expression of Pso p27 positively correlates with disease activity

Immunofluorescence microscopy of skin biopsies revealed Pso p27 positive cells in every psoriatic skin lesion investigated while no fluorescence was detectable in uninvolved psoriatic skin [21–23]. This demonstrated that Pso p27 was not a common skin protein but rather a locally expressed antigen (Fig. 4). The monoclonal mouse anti-Pso p27 antibodies were shown to be highly specific [22–25] and represent powerful tools for semi-quantification of Pso p27 [24]. When following patients treated with cyclosporin A, we observed a positive correlation between response to treatment and diminishing concentrations of Pso p27 in the selected skin lesions [22]. Equivalent results were obtained when patients were treated with herbal extracts according to traditional Chinese medicine [23] as exemplified in Fig. 5. Both studies were performed double blinded with semi-quantifying the prevalence of Pso p27 positive cells in the biopsies. Spontaneous fluctuations in disease activity also correlate with expression of Pso p27.

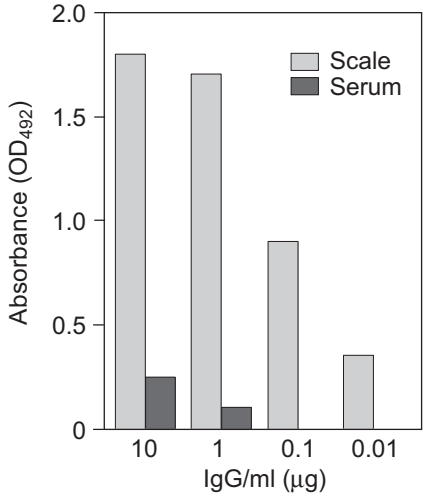

Fig. 2. Comparison of anti-Pso p27 antibodies in psoriatic scale extract and serum from the same patient demonstrated by indirect ELISA [18].

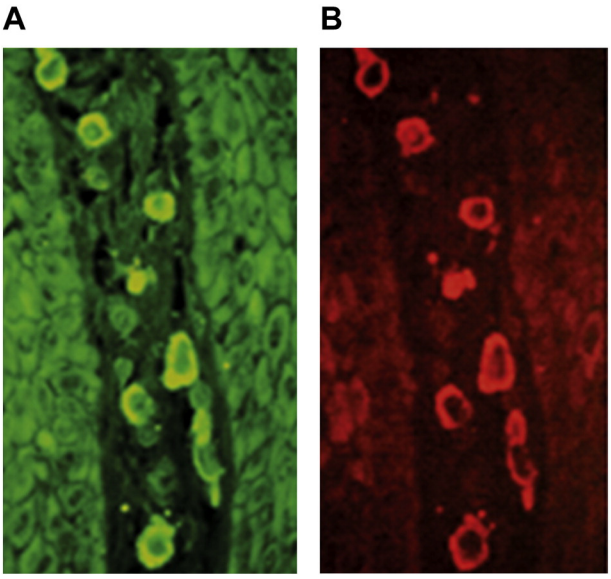

Fig. 3. Binding of scale antibodies (A) and rabbit anti-Pso p27 antibodies (B) to a thin section of a psoriasis lesion demonstrated by indirect immunofluorescence microscopy [19].

5. Identification of Pso p27 positive cells as mast cells

Infiltration of inflammatory cells is a characteristic feature of psoriatic lesions [26] and it was expected that the Pso p27 positive cells were among these cells. In an approach to characterise the Pso p27 positive cells, double labelling with anti-Pso p27 antiserum and monoclonal antibodies against specific surface membrane proteins on various immune cells was performed without coincidence. After elimination of lymphocytes and monocytes as candidates, we finally combined anti-Pso p27 antiserum and an enzyme assay specific for mast cell associated tryptase (Fig. 6). Given entirely concomitant staining for tryptase in the Pso p27 positive cells, we concluded that these cells were mast cells [21]. The absence of Pso p27 in mast cells in uninvolved psoriatic skin suggested that the presence of Pso p27 was a unique characteristic of the mast cells in the skin lesions [21].

These observations indicated a key role of mast cells in the generation of Pso p27 and substantiated the presumption of Pso p27 as an important antigen in the pathogenesis of psoriasis.

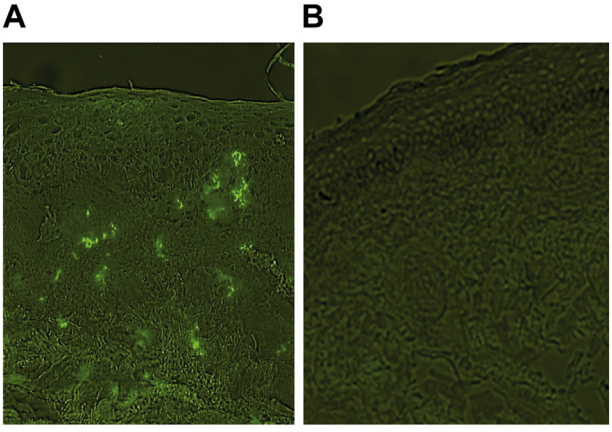

Fig. 4. Binding of specific monoclonal mouse antibodies against Pso p27 to thin sections of a psoriatic lesion (A) and uninvolved skin (B).

6. Pso p27 — a posttranslational modification of SERPINB3/B4

To map out the mechanisms underlying generation of Pso p27, we proceeded to sequence the protein by N-terminal Edman degradation [27]. The sequence showed homology with squamous cells carcinoma antigen (SCCA), and this relationship was strengthened by Yu et al. who demonstrated that a monoclonal antibody against Pso p27 bound to an epitope on SCCA2b [25]. Due to technological and methodological progress within mass spectrometry during the last decades, sequencing of larger peptides and proteins has improved considerably, and this opened up for more comprehensive characterisation of Pso p27. However, despite access to extensive quantities of Pso p27 from psoriatic scale, purified through immune-chromatography and preparative SDS-PAGE, analyses of the sequence data gave unexpected challenges due to the presence of non-canonical amino acids. The underlying reason for this is likely that Pso p27 was isolated from skin, which had been exposed to oxygen and ultraviolet light and leading to abundant protein oxidation. When this was accounted for in the database searches we found that the amino-acid sequence of Pso p27 shared homology with distinct sequences in both SERPINB3 (SCCA1) and SERPINB4 (SCCA2) [28]. Pso p27, which is a smaller protein compared to the native serpin molecules, was found to represent a well-defined core structure of SERPINB3/B4 lacking the N-terminal and C -terminal ends. Based on these findings we postulated that Pso p27 is generated through specific enzymatic digestion of the serpin molecules by highly specific endopeptidases [28]. The concomitant presence of Pso p27 and SERPINB3/B4 in mast cells in psoriatic lesions [29] established the mast cell specific endoproteases chymase and tryptase as candidates in catalysing the transformation of SERPINB3/B4 to Pso p27.

7. Generation of Pso p27 from recombinant SERPINB3

Overexpression of SERPINB3/B4 in epidermal cells in psoriatic lesions has been demonstrated by Takeda et al., [30]. However, as shown in Fig. 7, SERPINB3 is also present in dermal cells.

To examine whether Pso p27 could be generated from serpins by mast cell proteases, we employed purified recombinant full-length SERPINB3. Whereas no cleavage of SERPINB3 was observed when incubated with mast cell tryptase, three fragments were generated after treatment with mast cell chymase. Here, the largest fragment was identical to Pso p27 and two additional fragments represented the N-terminal - and the C-terminal ends of SERPINB3 [31] (Fig. 8A).

X-ray crystallographic analyses demonstrated that the N-terminal and the C-terminal fragments remained associated with Pso p27, forming a Pso p27-complex (Fig. 8B) with conspicuous structural similarities to SERPINB3 (Fig. 8C,D). However, despite the conformational similarities between the Pso p27-complex and SERPINB3, several distinct physicochemical and antigenic differences were found: 1) Unique monoclonal antibodies against Pso p27 did not recognise corresponding epitopes on SERPINB3 [29,31,32]. 2) The Pso p27-complex formed large aggregates compared to SERPINB3 [32]. 3) A polyclonal antiserum against the N-terminal end of SERPINB3 did not bind to the Pso p27-complex *in vivo* or *in vitro* [32]. The latter suggests that in the aggregated Pso p27 complex, the antibody-binding epitope is hidden by a highly ordered structure organization [32].

Based on these observations, we concluded that Pso p27 is generated from SERPINB3/B4 by enzymatic digestion with mast cell chymase. The three fragments remain associated in a Pso p27-complex in which the core subunit has an extended β-sheet conformation and that has a propensity to form large aggregates with defined structures, thus resembling the mechanistic foundation of many serpinopathies [33].

8. Serpins are metastable protease inhibitors

Serpins, of which 37 have been described in humans [34], are serine- or cysteine protease inhibitors that regulate a wide range of

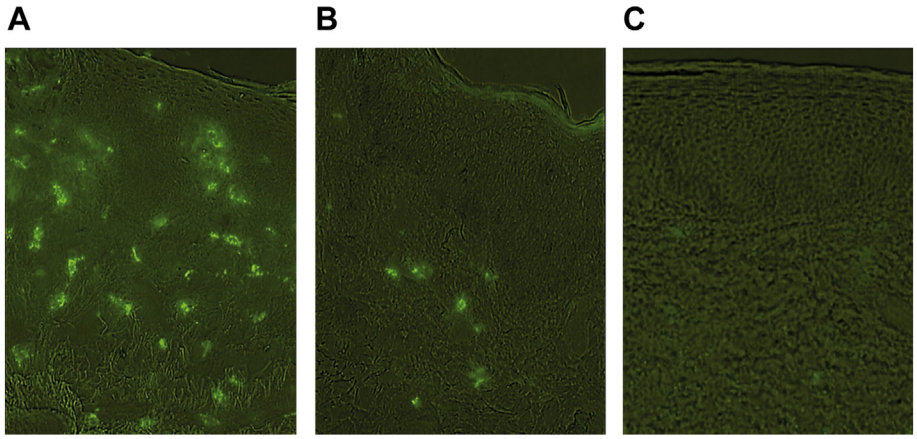

Fig. 5. Pso p27-expression in biopsies from psoriatic lesions before (A), and after (B, C) treatment with herbal extract demonstrating both various response to treatment and correlation between Pso p27 expression and disease activity.

physiopathological processes, including coagulation and inflammation, and are also involved in angiogenesis and apoptosis [35]. They share a common three-dimensional structure composed of three β-sheets (A-C), 8 or 9 α-helices and a flexible reactive centre loop (RCL) that serves as a pseudo-substrate for their target proteases. In the unbound condition, serpins exist in a metastable, high-energy state, and the energy relieved by interaction with the target proteases is clue to their inhibition. In the standard inhibitory mechanism of serine proteases, residues in the RCL of the serpin match with residues in the active site of the target protease. Upon binding, a scissile (P-P1′) bond of the RCL is cleaved by the protease leading to the formation of a stable acyl-enzyme intermediate where the protease forms an ester bond with the C-terminal of the cleaved RCL. The new C-terminal of the loop then inserts as an additional antiparallel strand into β-sheet A and translocates the protease to the opposite pole of the serpin molecule, thereby crushing the protease against the serpin surface rendering both the serpin and the protease irreversibly inactivated [36]. Some cross-class serpins targeting cysteine proteases may deviate from this behaviour. Thus, when SERPINB3 was incubated with papain, a stable covalent complex was not formed [37]. Moreover in the same study papain partially regained its activity when all SERPINB3 was cleaved SERPINB3 and SERPINB4 belong to the clade B serpins that encompass 13 human members [38]. They were originally purified from squamous cell carcinoma tissue, as the major component of the TA-4 antigen [39]. The two proteins are co-expressed in many normal tissues, including several areas of the skin, and elevated levels have been found in many epithelial malignancies [40,41] [Reviewed in [42]].

The SERPINB3 and B4 polypeptide chains both contain 390 residues and share an overall 92% sequence identity except in the RCL loop, in which the identity drops to 63% (Fig. 9). The differences in the RCL loops also underlie the target specificities of the two serpins. Whereas SERPINB3 has been shown to inhibit papain-like cysteine proteases such as papain and cathepsin S, -K and -L [43,44], SERPINB4 inhibits both cysteine proteases [45] and serine proteases [46].

Based on target-enzyme specificity of other inhibitory serpins, the RCL of SERPINB3 was originally predicted to harbour Ser-Ser at P1 and P1′ [47]. Notably, this cleavage site does not conform well to the substrate preference reported for mast cell chymase, which strongly prefers an aromatic residue at P1 [48]. Both Pso p27 isolated from psoriatic skin and Pso p27 generated by cleavage of recombinant SERPINB3 reveal two unique cleavage points at Tyr^{73}/His^{74} and Val^{349}/Val^{350} (Fig. 9). These cleavages should occur in both serpins *in vivo*, since peptides distinct to either serpin were found in Pso p27 isolated from psoriatic plaque [28]. The N-terminal cleavage site (Tyr-His) present in both SERPINB3 and B4 conforms well to the reported preference of an aromatic residue at P1, whereas His at P1′ was not reported in a previous study [48]. Thus

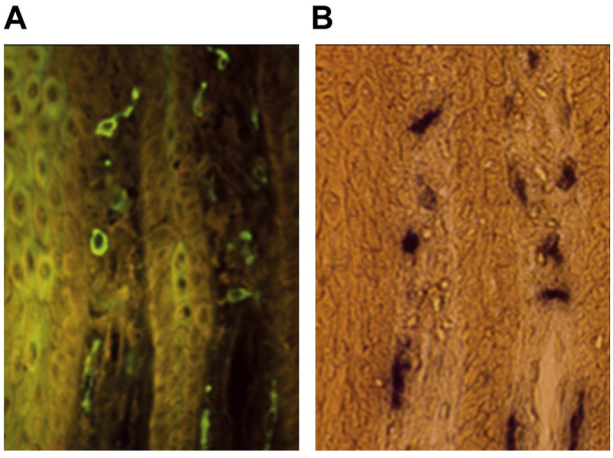

Fig. 6. Coincidental detection of Pso p27 positive cells (A) and tryptase containing cells (B) in a thin section from a psoriasis lesion strongly indicates that the Pso p27 cells are mast cells.

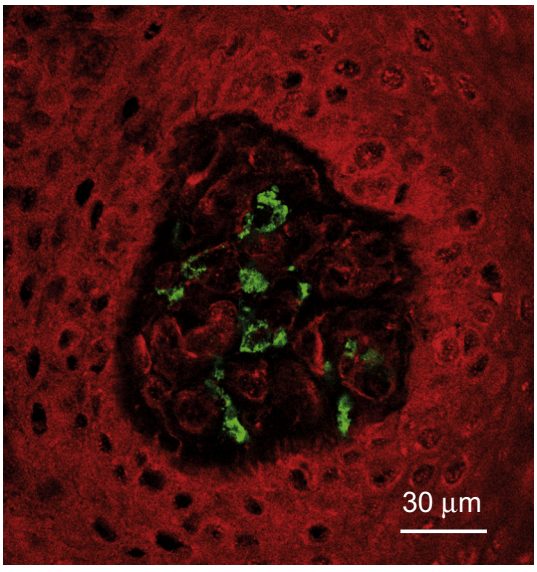

Fig. 7. Confocal image displaying a crosscut papilla of a psoriatic lesion with SERPINB3/B4 positive cells (red) and Pso p27 positive cells (green).

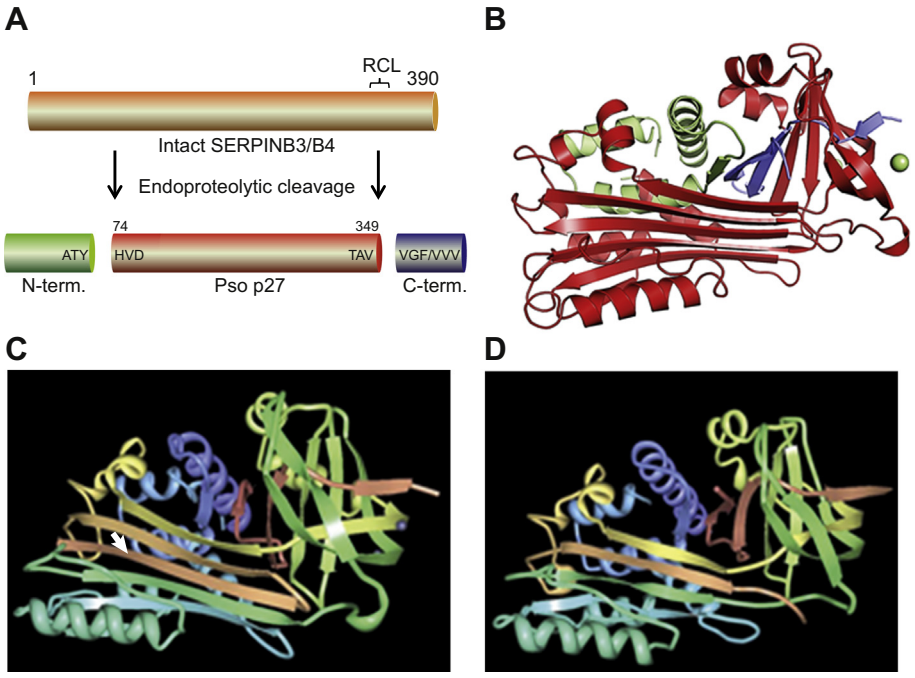

Fig. 8. A) Cleavage of SERPINB3/B4 by mast cell chymase yields a core fragment (red) identical to Pso p27 isolated from psoriatic scale, and N-terminal (green) and C-terminal (blue) fragments. B) The X-ray crystallographic structure of chymase-treated SERPINB3 revealed that the N- (green) and C-terminal (blue) fragments remained bound to the core fragment (red) in a Pso p27 complex. Residues highlighted in the C-terminal fragment are from the cleavage border in SERPINB3 (VGF) and SERPINB4 (VVV). Bottom panels: Cartoon illustrations of the Pso p27 complex (C) and SERPINB3 (D). The white arrow in (C) highlights the cleaved RCL inserted as a novel antiparallel strand in β-sheet A.

the substrate preference of mast cell chymase appears to be broader than previously reported.

Regulatory mechanisms are likely in place to avoid the apparent non-specific cleavage of SERPINB3/B4 *in vivo*, *e.g.* by physical separation of the chymase and serpins under normal circumstances. Our immunofluorescence analyses revealing absence of Pso p27 in mast cells in normal skin indicate that SERPINB3/B4 are not present in these cells. This was very recently supported by a comprehensive proteomic study of human skin mast cells in which 17 serpins were identified, but not SERPINB3/B4 [49]. Conversely, the same study robustly identified SERPINB3 in human dermal fibroblasts and both SERPINB3 and SERPINB4 in epidermal keratinocytes.

In summary, the above studies suggest that SERPINB3/B4 are not natural targets for mast cell chymase *in vivo*, Nevertheless, both serpins contains motifs that can be cleaved to produce Pso p27. Thus, the serpins and the mast cell chymase are likely spatially separated in healthy tissues.

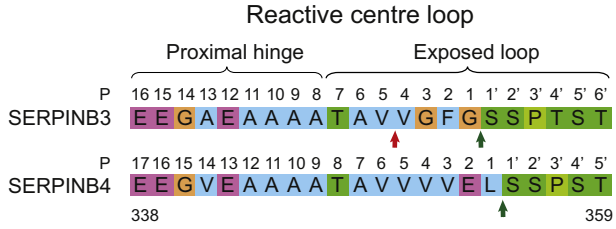

Fig. 9. The RCLs in SERPINB3 and SERPINB4 encompassing residues 338 to 359 in both proteins. Individual residues are coloured according to ClustalX [63] colour coding. Green arrows indicate canonical cleavage sites associated with protease inhibition in SERPIN B3 [64] and SERPINB4 [46]. Red arrow indicates (abnormal) cleavage of SERPINB3 by mast cell chymase, which does not mediate formation of a covalent intermediate.

9. Mast cells may play a crucial role in mediating an autocrine inflammatory loop

The underlying mechanisms causing SERPINB3/B4 overexpression in psoriatic lesions remain poorly understood, but local production of pro-inflammatory cytokines [50] most likely plays an important role. At least three of the cytokines found to be overexpressed in psoriatic skin, TNF-α [56] IL22 [57] and IL-6 (58), have been shown to promote increased levels of SERPINB3/B4.

The concomitant presence of intact SERPINB3/B4 and Pso p27 in mast cells [27,44] suggests that the transformation primarily takes place within the mast cells. The mechanisms whereby SERPINB3/B4 are recruited into the mast cells from the surrounding cells in psoriatic skin presently remain unknown. To the best of our knowledge, no receptors have been described that mediate cellular uptake of SERPINB3/B4. However, the low density lipoprotein receptor-related protein (LRP) is able to bind and internalize a wide range of structurally diverse proteins, including serpins and their protease complexes and activated forms of the pan proteinase inhibitor α2-macroglobulin (α2M) [51,52]. A high-affinity site for binding to LPR1 has been characterized, and consists of a cluster of three Lys and one Arg residue. It is noteworthy that SERPINB3/B4 contain several such basic clusters and their potential to bind LRP1 thus rewards further examination. LRP1 is amply expressed by human dermal fibroblasts, and to a lesser extent by skin mast cells [49]. We might thus speculate whether the high concentrations of SERPINB3/B4 in psoriatic lesions [30] by itself may cause endocytosis into the skin mast cells.

After being processed by mast cell chymase, the generated Pso p27-complexes show a tendency to form large aggregates and they may be released from the mast cell through a budding process or by cellular destruction (Fig. 1). Even if the molecular masses of the three peptide-fragments in the Pso p27-complex deviate somewhat from the estimated molecular masses of the internal proteins in the membrane coated virus-like particles obtained from psoriatic patients [13], these

may in fact represent aggregated Pso p27 complexes released from mast cells as budding particles.

The immunogenic potential of Pso p27 results in generation of antibodies and complement activating immune-complexes. This contributes to the inflammation through recruitment of inflammatory cells, release of various cytokines [53,54] and inflammation associated hypoxia [55,56] which will stimulate further expression of SERPINB3/B4. Serpin molecules will in turn be taken up by mast cells and in this way be substrate for generation of more Pso p27-complexes and generate a positive feedback loop that may explain the chronic inflammatory reaction in the psoriatic skin lesions. Such a positive feedback loop is modelled in Fig. 10. Biological agents against cytokines, such as anti-TNF antibodies, have been shown to suppress the disease activity in various inflammatory diseases. As TNF is shown to stimulate synthesis of SERPINB3/B4 we would expect the anti-TNF antibodies to inhibit this stimulation and consequently suppress generation of the autoantigen Pso p27.

10. A role of Pso p27 in other autoimmune diseases

Mast cells are supposed to play a key role in atopic dermatitis, in which SERPINB3/B4 have been shown to be upregulated related to disease activity [57]. Moreover, Hammeren identified Pso p27-containing cells in the skin lesions of atopic dermatitis [58] (Fig. 11). This correlates with the observation made in psoriasis. It is thus reasonable to hypothesize that the mechanisms for generation of Pso p27 are similar to that of psoriasis. Furthermore, Pso p27 protein has been detected in effected organs in various diseases like psoriasis arthritis [59], rheumatoid arthritis [59], ankylosing spondylitis [59], chronic inflammatory bowel diseases [17] and sarcoidosis [11,24] and indirectly through detection of anti-Pso p27 antibodies in cerebrospinal fluid from patients with degenerative disc disorders [60].

Overexpression of serpin molecules has been demonstrated in association with many inflammatory diseases including cancer. It is tempting to reflect whether generation of Pso p27 and the positive feedback mechanism modelled in Fig. 10 contribute to sustaining inflammatory reactions in various diseases even if the inducing factors are different. In support of this, a very recent meta-analysis that integrate RNAseq

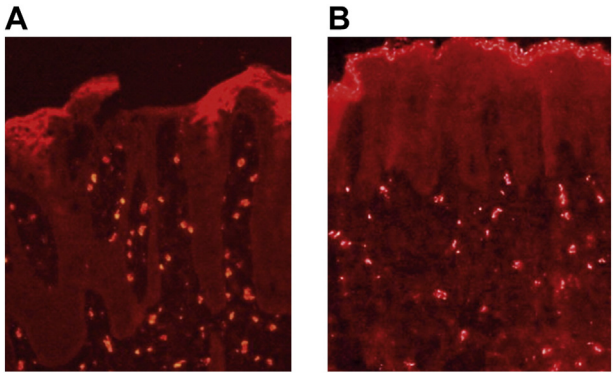

Fig. 11. Detection of Pso p27 in skin lesions from patients with psoriasis (A) and atopic dermatitis (B), using monoclonal mouse antibodies [58].

data from PBMCs of psoriatic patients, healthy individuals and patients suffering from a wide range of other autoimmune diseases such as MS, sarcoidosis and juvenile rheumatoid arthritis, most closely mirrored the differentially expressed genes in psoriasis [61].

Concluding remarks

If generation of the autoantigen Pso p27 is a common principle in chronic inflammatory diseases, mediating a feedback cyclic process as outlined in Fig. 10, interference in this process will open up for specific therapeutic strategies. Suppression of inflammation in general *e.g.* by TNFα inhibitors will likely interfere with the generation of SERPINB3/B4. However, such inhibitors are not specific and mediate substantial adverse effects [[62] and references therein]. Conversely, inhibition of SERPINB3/B4 uptake into the mast cells or inhibiting the aberrant cleavage reaction would represent specific approaches with potentially less adverse effects. Preliminary results from our laboratory indicate that inhibitors of the cleavage reaction and formation of Pso p27 is strongly inhibited by factors present in extracts of Chinese herbs used in traditional treatment of psoriasis. Such extracts are thus promising sources

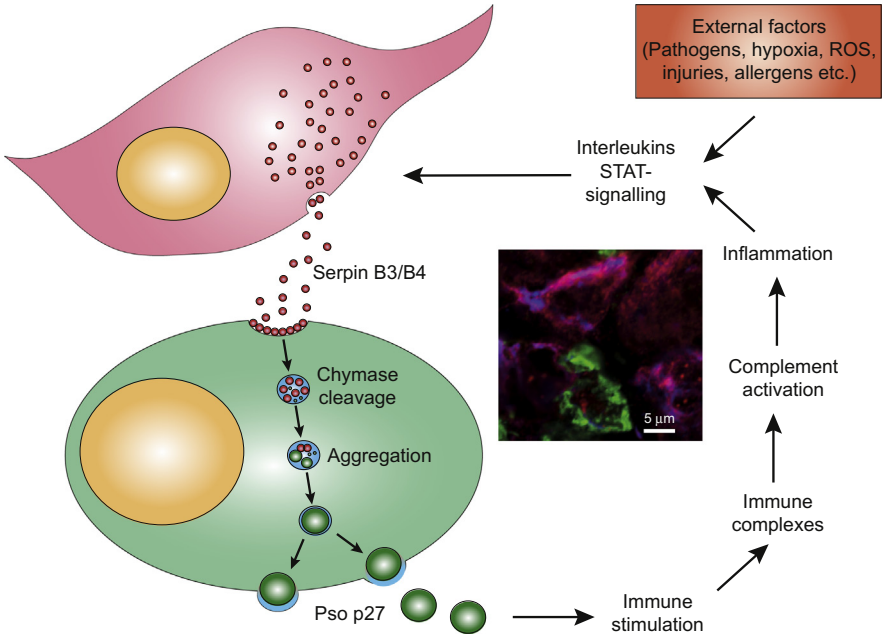

Fig. 10. Schematic model of the posttranslational modification of SERPINB3/B4 synthesised in epithelial cells, transformed to Pso p27 in mast cells, and releasedas Pso p27-complex aggregates in subcellular virus-like particles. A segment of the confocal micrograph image in Fig. 7 Demonstrates proximity of a SERPINB3/4-containing cell and a Pso p27-containing mast cell. (ROS; Reactive oxygen species).

of identifying drug candidates or lead molecules for targeted therapy of psoriasis and potentially also other autoimmune diseases.

Acknowledgement

We would like to thank The Cellular and Molecular Imaging Core Facility (CMIC), at NTNU, for performing the confocal microscopy of a psoriatic skin lesion (Fig. 7).

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