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Expression of the membrane complement regulatory proteins (CD55 and CD59) in human thymus

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Abstract: CD59 is one of the key molecules involved in cell protection against autologus complement. The fact that complement regulatory proteins are able to prevent hyperacute rejection of organs in pig to primate model, raises the question of possible complement regulatory protein (CRP) involvement in the maturation of immunological system. We report here that in foetal and postnatal human thymus, CD59 and CD55 are primarily located on Hassall's corpuscles and medullary epithelial cells. This localization highly correlates with the expression of CD30L, which is the member of the tumour necrosis factor superfamily. Additionally, TUNEL technique was used to visualize distribution of apoptotic cells in the thymus, which revealed the presence of apoptotic cells closely associated with the Hassall's corpuscles. The observed co-localization of CD59, CD55 and CD30L might suggest an involvement of the complement system in thymic selection in humans.

Key words: Hassall's corpuscles - CD59 - CD55 - CD30L - Immunohistochemistry - Complement - Apoptosis - Clone selection

Introduction

The membrane attack complex inhibitory protein CD59 and Decay Accelerating Factor CD55 are two of the key members of the family of complement regulatory proteins (CRPs), molecules protecting autologus cells against inappropriate complement activation [10]. Protection effect is achieved by interfering with the assembly of the membrane attack complex and the insertion of complement 9 into the cell membrane [33], thus preventing formation of membrane attack complexes (MAC). CD59 binds to preformed C5b-8 complex in a way that blocks polymerisation of C9 molecules forming the channel of MAC. Additionally to complement regulatory function, CD59 acts as a signal transduction molecule [11]. The primary role of CD55 is deactivation of complement (C3and C5) convertases [31]. Both CD59 and CD55 proteins are attached to the external surface of the cell membrane by glycosylphosphatidylinositol (GPI) moiety attached to their C-terminal end enabling high mobility of the molecule within the biological membranes. The molecules acting on two different levels of complement activation are very effective elements of anti-complement shield. The ability of CD59 and CD55 to stop the complement-mediated damage has been used to develop strategies aimed at creation of transgenic animals which serve as organ donors for clinical applications [1, 7-9, 12, 18, 26, 27, 29, 35]. The observed protective effect preventing rejection of xenotransplants raises the question about possible involvement of CRPs in the selection of lymphocytes in human thymus.

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Uncontrolled overexpression of CD59 and CD55 on thymocytes might result in decreased sensitivity of these cells to complement-mediated damage and in turn protect them from immunologic surveillance, as demonstrated in case of several malignant tumour cells [16, 30, 48]. As leukaemic blasts are protected by relatively high expression of CRPs [17], the precise regulation of GPI-anchored CRPs distribution in thymus seems very essential for the normal function of this organ. Numerous investigators have extensively studied the distribution of CD59 proteins in the human organism [2, 20-23, 28, 32, 36, 37, 42, 47] but there is almost no information available about its morphological localisation in thymus. The aim of the present study was to elucidate the distribution of CD59 in human thymus.

Materials and methods

Materials and reagents. The study was performed on 9 thymus samples obtained from humans of different age. Thymuses were obtained as surgical waste material removed during corrective heart surgery. Three of them were foetal (age 6-8 months of pregnancy) obtained during autopsy. The other six thymuses were obtained during surgery (age 0-13 years of age). CRPs were localized in standard paraffin sections using immunohistochemical methods. Indirect biotin-avidin-peroxidase technique with two different anti-human CD59 monoclonal antibodies: rat IgG clone YTH.53.1 (Serotec® Kidlington, UK) and mouse IgG Clone MEM-43 (Cymbus, Hampshire, UK). CD55 was identified with mouse monoclonal antibodies (Serotec® Kidlington, UK). CD30L antibody was purchased from Genzyme Diagnostics® (Cambridge, MA). Macrophages were localised using monoclonal antibody NCL-LN5 (Novocastra® Newcastle upon Tyne, UK). Thymocytes were identified by staining with mouse anti CD3 monoclonal antibodies (Novocastra® Newcastle upon Tyne, UK). Apoptotic cells were localised using TUNEL technique (ApopTag™ Oncor®, Gaithersburg, MD).

Immunohistochemistry. The thymic tissue was fixed in standard 4% paraformaldehyde aqueous solution and embedded in paraffin. Staining was performed on 8 µm paraffin sections which were first heated at 56°C for 60 min and then deparaffinised. Additionally, high temperature unmasking technique was used. In brief, slides were boiled in 0.01 M sodium citrate buffer (2.94 g/l of tri-sodium citrate solution adjusted to pH 6.0 using 1.0 M HCl) in a pressure cooker for about 5 min. After the heat treatment, slides were washed in TBS (Tris buffered saline). After quenching endogenous peroxidase with 0.5% H_2O_2 in methanol for 30 min, sections were blocked with horse serum for 30 min at room temperature and exposed to monoclonal antibodies at 4°C overnight. Staining was performed using anti-CD59 monoclonal antibodies (rat YTH 53.1 and mouse MEM43) (1:100) anti-CD3 antibodies, NCL-LN5 antibodies (1:50) and anti-CD30L antibody (1:50). Visualisation of antibodies was obtained using ABC technique according to manufacturer's protocol (Novocastra ABC kit, cat. no. NCL-ABCu). DAB (Sigma®) was used as chromogen. Sections were counterstained with haematoxylin and mounted in glycerol-gelatin (Sigma®). Isotypic antibodies (Serotec® Kidlington, UK) were used as a control.

TUNEL method. Detection of apoptotic cells was performed using commercial kit (ApopTag™ (Oncor®, Gaithersburg, MD), according to manufacturers recommendations. Briefly, after deparaffinization slides were incubated in 1 µg/ml of Proteinase K in 10 mM Tris solution for 15 min at room temperature. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide in phosphate buffered saline (PBS). The sections were then incubated with terminal deoxynucleotidyl transferase at 37°C for 60 min (adding the digoxigenin-conjugated dUTP to the 3'-OH ends of fragmented DNA). To detect the digoxigenin-labelled nucleotides, specific antibodies conjugated with peroxidase were used. The sections were stained with 4-chloro-1-naphtol (CN). Apoptotic cells were stained dark blue.

Results

In foetal thymus (3 cases examined), CD59 was identified predominantly on Hassall's corpuscles (Fig. 1) in the medulla, decreasing its expression during the first year after birth (3 cases). In thymuses obtained from older patients (more than 1 year old, 3 cases), CD59 was still detectable on Hassall's corpuscles (Fig. 3A) but only when high temperature unmasking technique was used. The distinctive feature of the staining was the multilayered pattern of Hassall's corpuscles (Fig. 2). The staining pattern was similar for anti-CD59 (YTH.53.1 and MEM 43) and anti-CD55 monoclonal antibodies used in the study (Fig. 3B). The results of TUNEL staining revealed accumulation of apoptotic cells in close contact with the Hassall's corpuscles (Fig. 3C). Apoptotic cells were also distributed "randomly" in other parts of thymus (not shown). Staining with anti-CD30L antibody revealed distribution of this protein mainly on Hassall's corpuscles, which was similar to that of CD59 and CD55 (Fig. 3D). A different pattern of staining was observed when the sections where labelled with monoclonal anti-CD3 antibodies (Fig. 3E) or macrophage marker (LN5 MoA) (Fig. 3F). In both cases Hassall's corpuscles were negative, while positive cells (CD3 and LN5) were present in large numbers in thymic medulla and cortex. Isotypic controls revealed no unspecific binding of antibodies to Hassall's corpuscles. Additionally it was observed that CD3-positive thymocytes and LN5-positive phagocytic cells had low level of CD59 and CD55 expression. The level of CD59 and CD55 expression on thymocytes increased with age and was dependent on the region of thymus. It was not detectable in cortex and increased toward the medullary region.

Discussion

Despite the widespread interest in thymus development [4-6, 39], relatively little is known about the role and origin of Hassall's corpuscles. These structures consisting of several layers of cell membranes are frequently found in the thymic medulla of thymus. The number of Hassall's corpuscles increases with age, indicating their involvement in thymus maturation. Additionally, the morphology of Hassall's corpuscles changes with age: in foetal thymuses they are relatively small and regularly shaped in contrast with struc-

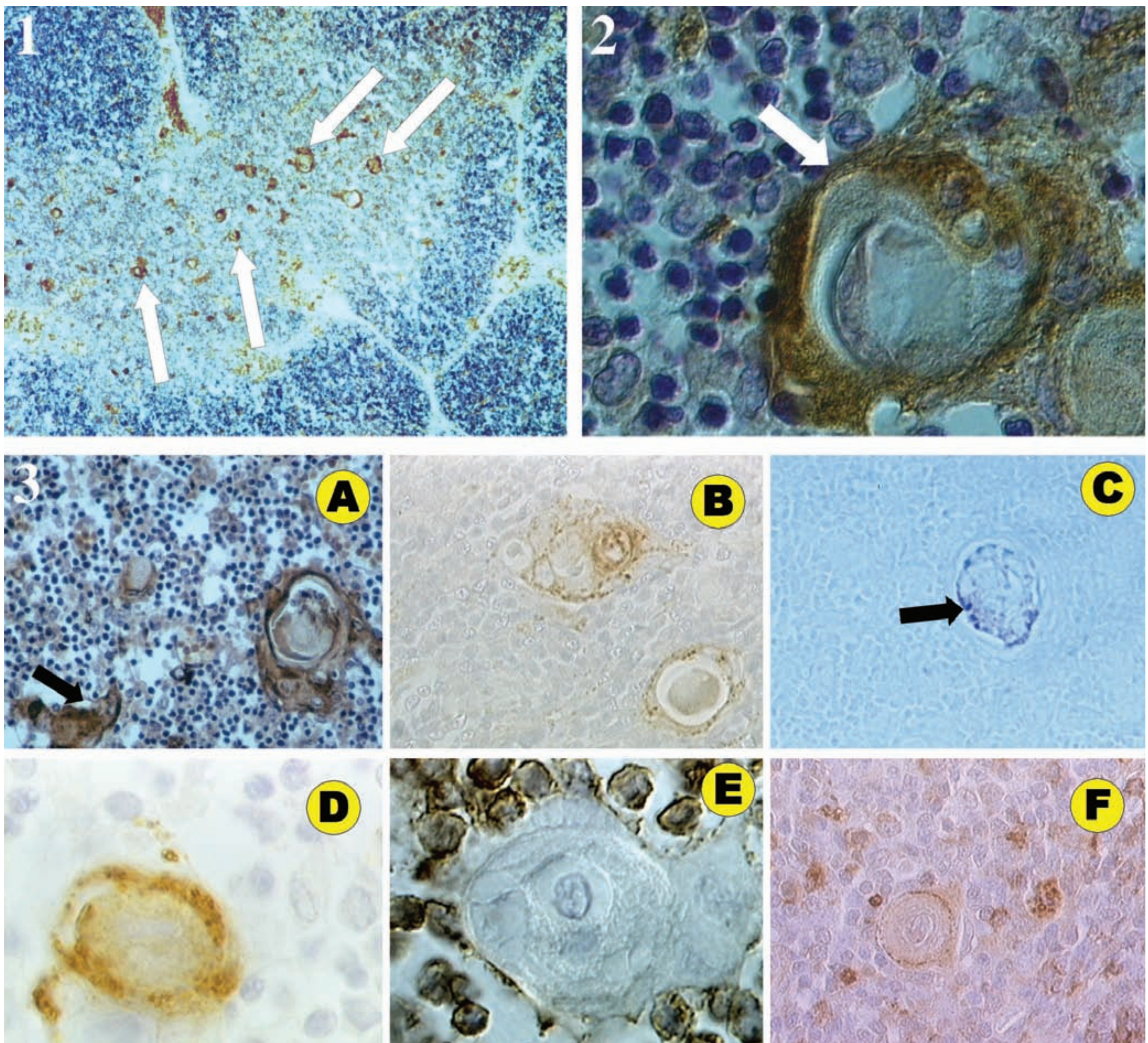

Fig. 1. CD59 distribution in foetal human thymus. Arrows are pointing to Hassall's corpuscles. × 100. **Fig. 2.** CD59 localization within Hassall's corpuscles from foetal human thymus. Clearly visible association of CD59 with membranes (arrow). × 600. **Fig. 3. A**. CD59 localisation in human postnatal thymus on Hassall's corpuscle and nursing cells (arrow). × 200. **B**. CD55 localisation on foetal Hassall's corpuscle. × 200. **C**. Apoptotic cells (indicated by arrow) in close association with outer layer of Hassal corpuscle. Postnatal thymus (TUNEL method). × 200. **D**. CD 30L immunostaining of Hassall's corpuscle. × 400. **E**. Thymocytes surrounding Hassall's corpuscle. CD3 immunostaining. × 400. **F**. Postnatal thymus stained with macrophage marker, antibody NL-5. × 200.

tures appearing during first years of life [38]. The pattern of CD59 staining in thymus was almost identical to that observed by other authors in case of CD30L [41]. This observation and the localization of apoptotic cells in close contact with CD59-positive cells, might suggest that those two molecules might play an important role in thymic selection in man. This hypothesis is also supported by clusterin distribution in rat thymus [13, 14]. Clusterin, which is analogous in function to CD59 in preventing MAC formation [45], is highly expressed in the medullary region of thymus [13, 14]. This localisation together with low level of CD59 on thymocytes indicates possible high susceptibility of immature thymocytes to complement attack. It is possible that CD59 expression increases on Hassall's corpuscles because of close association with apoptotic thymocytes in a similar way as proposed in case of clusterin [25, 40].

It might be also hypothesized that Hassall's corpuscles act as CD59 scavengers, collecting CRPs from apoptotic thymocytes. As outer layers of cells forming Hassall's corpuscles are closely associated with apop-

totic cells, it might be hypothesised that they act in a similar way as high endothelial venule cells [19].

The signal from CD30L transduced into cells via TNF receptors seems to induce not only apoptosis in negatively selected thymocytes, but also CD59 release from the cell surface, rendering thymocytes vulnerable to complement attack. Transfer of CRPs can be facilitated by the close physical association between thymocytes and stromal cells [43]. Decrease in CRP expression has been already observed during apoptosis of peripheral granulocytes [24] and lymphocytes [3, 23, 46]. On the other hand, increased expression of CRPs on lymphomas has been identified as an element of evading immunological surveillance [15, 34, 44]. It might be hypothesised that CD59-deficient thymocytes even when released from thymus might be eliminated from the organism by simple complement activation. iC3b-coated apoptotic vesicles might be phagocytosed by spleen and/or liver macrophages. This mechanism might explain the apparent inconsistency between death rate in thymus and the capacity of thymic macrophages to destroy cell debris. The observed increased surface expression of CRPs on medullary thymocytes might suggest that maturation of thymocytes might be associated with the acquisition of resistance to complement attack.

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