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Natural antiband 3 antibodies in patients with sickle cell disease

Anticorps naturels antibande 3 chez les patients drépanocytaires

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Band 3 oligomers, precociously formed in the membrane of sickle red blood cells (SS RBC) as a result of oxidative damage, induce two significant changes: (1) contribution to the adhesive nature of these cells to endothelial cells; (2) production of recognition sites for natural antiband 3 antibodies (antiband 3 Nabs). The inhibition of the adhesion of SS RBC to endothelial cells by band 3 peptides suggests a participation of antiband 3 Nabs in the etiology and prevention of vaso-occlusive crises (VOC). To address this question, we measured the levels of antiband 3 Nabs in sickle cell anaemia (SCA) patients (45 in steady state, 35 in VOC) and in controls (27 sickle trait, 30 normal AA subjects). A significant decrease of antiband 3 Nabs in the VOC group was demonstrated as compared with the steady state group, the sickle trait and healthy controls. This study provides data suggesting that Antiband 3 Nabs are likely to play a role in the SCA VOC.

R É S U M É

Les agrégats de bande 3 formés dans la membrane des globules rouges drépanocytaires (GR SS), du fait d'un stress oxydant, induisent des modifications d'importance à deux niveaux : (1) augmentation des propriétés d'adhérence des GR SS aux cellules endothéliales ; (2) production de sites de reconnaissance pour les Antiband 3 Nabs (anticorps naturels antibande 3). L'inhibition de l'adhérence des GR SS aux cellules endothéliales, par des peptides issus de la bande 3, suggère une participation de ces anticorps dans l'étiologie et la prévention des crises vaso-occlusives (CVO). Pour tester cette hypothèse, nous avons mesuré les taux d'antiband 3 Nabs de patients drépanocytaires (45 à l'état de base, 35 en CVO) et de contrôles (27 sujets AS et 30 AA). Une diminution significative des taux de Antiband 3 Nabs a été retrouvée pour les patients en situation de CVO comparativement aux patients en état stable et aux 2 groupes contrôles. Ces données suggèrent que, dans la drépanocytose, les antiband 3 Nabs participent au processus vaso-occlusif.


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1. Introduction

Sickle cell disease (SCD) is a severe and frequent genetic disease widespread mainly in Sub-Saharan Africa and therefore in regions where people are of African descent such as in the Caribbean. The disease is due to a mutation at position 6 of the β-globin chain (transversion A → T) resulting in the replacement of a glutamic acid by a valine and the appearance of an abnormal haemoglobin, haemoglobin S (Hb S, α2β2S). The polymerization of deoxygenated Hb S caused deformation (sickling) of the red blood cells that become rigid and fragile. Thus, SCD is characterized by chronic hemolysis and recurrent ischemia due to micro-vascular occlusion following the adhesion of erythrocytes and leukocytes to the activated vascular endothelium. Vaso-occlusion seems to be the common basis to all acute and chronic complications characteristic of this disease. Even though there are important advances in the current understanding of sickle cell vaso-occlusion, the basis of its control and prevention remain partially unknown. There is not an individual mechanism that could explain this phenomenon, its cause could be different from one to another event and its severity differs between patients [1–3]. Recent data suggest that vaso-occlusion involves complex circulating cell–cell interactions and circulating cell adhesion to the endothelium. Several investigations have allowed accumulating information about adhesion molecules in sickle cell red blood cells (SS RBCs) as well as in vascular endothelium that participates in the occlusion phenomenon [4–6]. These cellular interactions, delay the transit time of the SS RBCs in the small vessels and promote the polymerization of Hb S, the sickling of the RBCs and the occlusion in the microvascular lature [3]. These occlusions generate a vicious circle with a phenomenon like ischemia–reperfusion that induces an inflammatory response characterized by leukocytosis and expression by the endothelial cells of inflammatory cytokines, adhesion molecules [7,8]. This sequence of events is also a source of oxidative stress with production of free radicals.

In this pro-inflammatory environment, the RBCs are a target of reactive oxygen species produced by activated cells, including leukocytes. Moreover, the repeated cycles of SS RBCs sickling, coupled with the inherent instability of Hb S and deficient mechanisms against free radical production, are a second source of oxidative damage to the SS RBCs. Thus, compared to normal red blood cells (erythrocytes containing Hb A), SS RBCs generate about twice as reactive oxygen species (O2•−, H2O2, OH•) and oxidation products lipids and proteins [9,10]. This context of oxidative stress leads to severe defects in the erythrocyte membrane and particularly conformational modifications of the membrane protein band 3 and an abnormal exposure of phosphatidylserine (PS) [11–13]. The band 3 protein, which refer to a family of anion exchanger proteins present in the membrane of all cells and cellular organelles, is the main erythrocyte membrane protein and has been considered to be an important candidate to participate in erythrocyte-vascular endothelial interactions [12,14]. It is well-documented that during the red blood cell lifespan band 3 proteins aggregate in the human erythrocyte membrane surface, mostly due to oxidative insults that gradually accumulate [15–17]. In haemoglobinopathies such as SCD, the band 3 clusters are precociously formed as a result of the accelerated haemoglobin autooxidation, iron accumulation in membranes, increased membrane damage and a shorter red cell life span [9,18].

Aggregated band 3 on the SS erythrocytes produces two significant consequences: first, they provide to the RBCs novel adhesive sites for endothelium adhesion, as indicated by accumulated findings related to the effect of band 3 conformational changes due to oxidative insults [12,14]; second, they also provide binding sites for natural band 3 antibodies [17,19]. Data have shown that band 3 peptides are able to inhibit the adherence of sickle cells to endothelium (12). This suggests a possible participation of band 3 antibodies in the etiology as well as in the prevention of the painful crisis in SCD.

In this article, we test the hypothesis of the possible regulation of the sickle cell vaso-occlusive phenomenon by the natural band 3 antibodies by assessing the level of these antibodies in a group of SCA patients in steady state and during painful crisis and in control groups composed of healthy controls (AA) and sickle cell trait subjects (AS).

2. Material and methods

2.1. Patients

Eighty adult sickle cell anaemia patients (Hb SS), 33 females and 47 males, with a median age of 28 years (range 18–39 years), 45 in steady state, 35 during painful crisis admitted at the Institute of haematology and immunology were eligible for inclusion. A painful crisis was defined as pain in extremities, back, abdomen, chest or head without infections or other clinical complications. Steady state was defined as a period of at least 3 months previous to the study without transfusions or any clinical manifestations. Twenty-seven sickle trait individuals (Hb AS) and thirty healthy controls were recruited as reference groups (Hb AA). All patients and controls gave written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki.

2.2. Band 3 antibodies measurement

Blood samples were taken from the antecubital vein and collected into 10.0 mL dry tubes. Within 45 min after collection, sera were obtained by centrifugation (20 min at 1550 × g at 4 °C). Sera aliquots of 0.50 mL were immediately stored at −20 °C.

Natural band 3 antibodies, in sera of Hb SS patients, Hb AS and healthy Hb AA controls, were measured by an enzyme immunoassay using band 3 covalently bound to NUNC CovaLink NH microplates, as described in [20] with slight modifications. Briefly, band 3 protein, purified from Triton X100 extracts of RBC membranes, using anion exchange and affinity chromatography, was covalently bound to NUNC CovaLink NH microplates. Aliquots of sera were incubated in the microplates with the immobilized
band 3. The detection of the band 3 antibodies was done by the mean of an incubation with anti-human IgG alkaline-phosphatase conjugate followed by an incubation in presence of its substrate, p-nitrophenylphosphate. Then, absorbance was measured at 405 nm in a microplate reader.

2.3. Statistical analysis

Results are presented as means ± SD. Differences between groups were tested by the Anova test and \( P \) values of 0.05 or less were considered statistically significant. Statistical analyses were performed using SPSS 11.5 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Comparisons of natural antiband 3 levels between sickle cell anaemia and control groups

There was a significant decrease in the levels of natural band 3 antibodies in the Hb SS patients with painful crisis in comparison with the Hb SS patients in steady state (Table 1). The antiband 3 levels in the group of patients with painful crisis were also significantly decreased as compared to the control groups of Hb AS and Hb AA subjects (Table 1).

4. Discussion

Band 3 clusters formed in the surface of senescent or oxidized erythrocytes are recognized by natural band 3 antibodies of the IgG class that are able to react with the complement system. This recognition leads to a band3/IgG/C3b complex and to the elimination of the labelled cells by macrophages in the spleen as part of the normal mechanism of elimination [17,21]. A premature senescence of the erythrocytes, due to haemoglobin S autooxidation and the inefficiency of the antioxidant defence, occurs in SCD [22]. This process can induce aggregation of the band 3 proteins giving to the erythrocytes the possibility to be recognized by natural band 3 antibodies [17] and/or to adhere to the vascular endothelium if the senescence epitopes are not masked by the IgG [12,14]. A decrease in antiband 3 levels due to, either an excess uptake due to a high level of adhesive cells, or a decrease of its production might promote the vaso-occlusion process.

In our study, we demonstrate a significant decreased of the levels of natural band 3 antibodies in the group of SCA patients with painful crisis compared with those in steady state, sickle trait patients and healthy controls. This finding suggests that the binding of band 3 antibodies to band 3 clusters in the membrane of SS erythrocytes could be a favourable mechanism to disable its adherence to vascular endothelium. The painful crisis could be produced when, due to different causes including an exacerbation of the oxidative stress, an excess of adherent SS erythrocytes is formed resulting to the decreased levels of natural band 3 antibodies. It seems that patients in steady state have sufficient amount of natural band 3 antibodies to eliminate the prematurely oxidized SS erythrocytes continuously formed and therefore masking the adhesive sites formed by the band 3 clusters. Levels of natural band 3 antibodies in sickle trait individuals were similar to that of normal controls.

In summary, our study has provided data suggesting that natural band 3 antibodies participate in the vaso-occlusion phenomenon. The possibility to regulate the levels of band 3 antibodies in SCD patients has an interesting therapeutic potential. Further studies are needed to define more precisely the role of antiband 3 antibodies in the pathophysiological mechanisms of SCA.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


