# Binding of $\alpha_1$-antichymotrypsin to the surface of lymphocytes – preliminary study

Sobieska M*

Department of Physiotherapy, Rheumatology and Rehabilitation, Karol Marcinkowski University of Medical Sciences, Poznań, Poland

## Abstract

**Purpose:** **The paper presents preliminary results of investigation of binding of $\alpha_1$-antichymotrypsin to the surface of peripheral blood lymphocytes.**

**Material and methods:** **Pooled serum samples from healthy individuals served as source of $\alpha_1$-antichymotrypsin for isolation using chromatography. Binding of $\alpha_1$-antichymotrypsin to the surface of peripheral blood lymphocytes was measured by flow cytometry.**

**Results:** **Even on native cells $\alpha_1$-antichymotrypsin may be detected. After incubation with isolated preparation the percentage of positive lymphocytes increased.**

**Conclusions:** **The presence of $\alpha_1$-antichymotrypsin on the surface of lymphocytes may imply its regulatory role during acute phase response and early immune response.**

**Key words:** $\alpha_1$-antichymotrypsin, lymphocytes, flow cytometry.

## Introduction

$\alpha_1$-antichymotrypsin (ACT) belongs to human serum $\alpha_1$-globulins. It is a glycoprotein, MW of about 68,000 Da, with 22.7% of carbohydrate content. It is encoded on chromosome 14, region q31-q32.3, the same area as antitrypsin gene. Both proteins are highly homologous, suggesting shared evolution history [1]. Biologic function of ACT is not entirely clear: it is a protease inhibitor, mainly of chymotrypsin, katepsin G and several others (to a lesser extent), however, much lower serum concentrations imply that its function as an antiprotease is rather a minor one [2]. Its role as an acute phase reactant appears much more important. In contrast to antitrypsin and alpha2-macroglobulin the concentration of ACT increases within 6 hours during acute phase response, similarly to CRP and SAA, making ACT one of the first line of acute phase reactants. It may also be involved in local regulation of homeostasis (or balance between proteases-antiproteases), among others in lungs, the predominant site of synthesis, and in brain, where it is synthesized by astrocytes [3].

In healthy individuals the serum concentration of ACT is 0.35-0.45 g/L. It increases in acute and chronic infections (mainly bacterial), in trauma, particularly in cases with massive necrosis or if polytrauma is accompanied by cranio-cerebral trauma [4]. There were also several reports that ACT is able to bind chromatin, similarly to CRP. It could thus participate in removal of cellular debris, preventing autoantibodies formation [5]. No homozygous deficiency of ACT was ever described, in contrast, only heterozygous deficiency clinically presented as decreased serum concentration [6,7].

There is a discrepancy in descriptions of glycans on ACT molecules: three or four glycosylations sites, bi- or triantennary glycans, consisting of NacGlc, Gal and Sia. If the glycosyaltion profile was investigated using crossed affinity immunoelectrophoresis with Concanavalin A as ligand, following variants are observed: first migrating, clearly separated, weakly reactive with ConA variant A1, variants A2, A3 and A4 hardly separated, but showing increasing reactivity with ConA. In particularly acute conditions and often in children a fifth variant (A5), almost non-migrating due to very strong reaction with ConA appears. Acute inflammatory conditions cause dramatic increase in ACT concentration and a shift towards biantennary glycans (higher reactivity with ConA). Particularly expressed changes were observed for cranio-cerebral trauma and burns with massive necrosis [8-10]. It seems that concentration of ACT reflects not the size, but the severity of damage of the

* CORRESPONDING AUTHOR:
Department of Physiotherapy, Rheumatology and Rehabilitation
Karol Marcinkowski University of Medical Sciences
ul. 28. Czerwca 1956 roku 135/147
61-545 Poznań, Poland
e-mail: magdula2811@poczta.onet.pl (Magdalena Sobieska)

Received 11.04.2005 Accepted 14.07.2005

central nervous system [11]. The prolonged (longer that one week) presence of the altered glycosylation profile is highly unfavourable prognostic factor, especially in patients with cranio-cerebral trauma [4].

All these data suggest that antichymotrypsin may exert an unknown biological role influencing lymphocytes during early stages of acute phase response. Some data were also obtained from observation of ACT behaviour in children suffering from food allergy. Decreased ACT concentration was observed in symptomatic children, whereas normalization of serum concentration accompanied tolerance [12]. To check this it seemed necessary to show that antichymotrypsin is able to bind with the surface of lymphocytes.

## Material and methods

Isolation of antichymotrypsin was performed using affinity chromatography: first simple ion exchange chromatography to remove albumin and majority of gammaglobulins, then on a column with coupled polyclonal anti-ACT antibody. Both columns were washed with 0.1 M phosphate buffer. ACT was isolated from serum pool from healthy persons, showing normal concentration and glycosylations profile of ACT. In this way a crude preparation was obtained, but the concentration of ACT was significantly higher in comparison to some trace proteins. The preparation was finally dialyzed against PBS (+4°C, overnight). The concentration of ACT was measured using rocket immunoelectrophoresis acc. to Laurell [13]. Determination of glycosylation profile of the preparation obtained was done using crossed affinity immunoelectrophoresis with ConA [14].

The very preliminary data was obtained from indirect staining. As primary antibody, DakoCytomation anti-ACT (Code number A0022) was used, as secondary antibody: anti-rabbit IgG, conjugated with FITC (DakoCytomation, Dennmark). As polyclonal antibody and indirect staining caused too many problems, and no conjugated anti-ACT antibody was available, it was decided to perform coupling of anti-ACT antibody with FITC, using Fluorotag Conjugation kit (FITC-1) from SIGMA, USA. First experiments were performed to detect ACT on the surface of peripheral blood cells, both native (without any procedure) and incubated first with ACT preparation. Peripheral blood samples from routine laboratory (drawn with addition of EDTA) were used for binding experiments.

Next set of experiments was performed to show the binding of ACT to CD3+ T lymphocytes and CD19+ B lymphocytes (in both cases antibodies from DakoCytomation, anti CD3, RPE-Cγ5, and anti-CD19, RPE-Cγ5, were used). DakoCytomation antibodies were used according to prescription: 5 microliters per 100 microliters of whole blood, and also 5 microliters of anti-ACT-FITC antibody was used. As previously, samples of native cells were stained in parallel with cells incubated with ACT preparation (mean concentration 100 mg/l, mixed 1:1 with whole blood, 1 hour in RT, then 3x washing with PBS).

## Results

The obtained anti-ACT/FITC conjugate had the concentration of 0.5 g/L and the molar protein/FITC ratio of about 0.8. First experiments using this antibody confirmed the presence of ACT to various extents on the surface of native leukocytes. Preincubation of whole blood with ACT preparation caused increase in the percentage of ACT positive cells, to various extents. Cells were gated as lymphocytes, monocytes and granulocytes acc. to routine forward scattering (FW-SC) versus right scattering (RT-SC), and only the percentage of positive cells (FITC – green fluorescence) was analyzed in each gate. Following controls were used:

- incubation of blood cells with another rabbit antibody;
- incubation of blood cells with normal rabbit serum;
- incubation of blood cells with normal goat serum;
- incubation of blood cells with secondary anti-rabbit $IgG^{FITC}$ antibody only;
- incubation of blood cells with primary unconjugated a-ACT antibody only.

In none of those controls the positive staining was present.

The results of the above measurement showed that even for limited number of experiments (n=7) the statistically significant difference was observed in t-test for dependent variables (p=0.000083 for lymphocytes, 0.000112 for monocytes and 0.02375 for granulocytes). Thus, ACT can be detected on the surface of peripheral blood cells and preincubation of blood cells with ACT preparation increased the percentage of ACT positive cells.

Next set of experiments (n=9) was performed to show the binding of ACT to CD3+T lymphocytes and CD19+B lymphocytes. The summary of results is presented on *Fig. 1* – double staining with anti-ACT (FITC) and anti-CD3 (PE), on the *Fig. 2* – double staining with anti-ACT (FITC) and anti-CD19 (PE).

## Discussion

It was detected that ACT is present not only on the surface of monocytes that produce it, on the surface of granulocytes that may engulf it in phagocytosis, but also on the surface of lymphocytes. This feature was not described before. Additionally it was shown that preincubation of lymphocytes with crude ACT preparation increased the percentage of lymphocytes bearing ACT on their surface and also that the intensity of staining with anti-ACT antibody increased after incubation. The main binding was observed on CD3- and CD19-lymphocytes, presumably NK cells.

Preincubation mimics naturally occurring increase of ACT at concentration in peripheral blood due to trauma or burn. In early acute phase response several mechanisms related to cellular activity are inhibited and mainly the unspecific immunity is involved in first stages of the reaction, also during unspecific inflammation occurring in Alzheimer's disease [15]. Thus it is possible that ACT may exert a regulatory role during these early events, possibly acting as anti-apoptotic agent [16]. Higher incidence of ACT on the surface of NK than T lymphocytes may

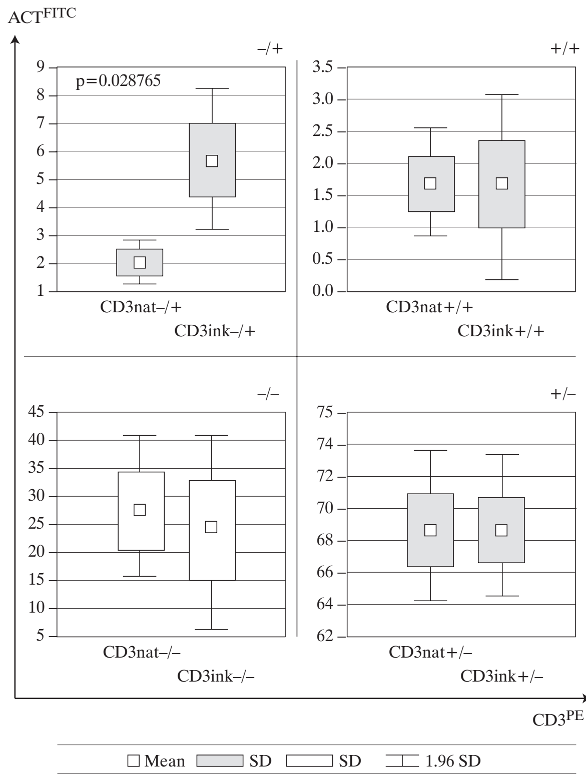


*Figure 1*. **Double staining (mean results for 9 experiments) with anti-ACT (FITC) and anti-CD3 (PE) – percentage of positive lymphocytes, native versus incubated with ACT preparation (point=mean, frame=SD, range=1.96 SD)**

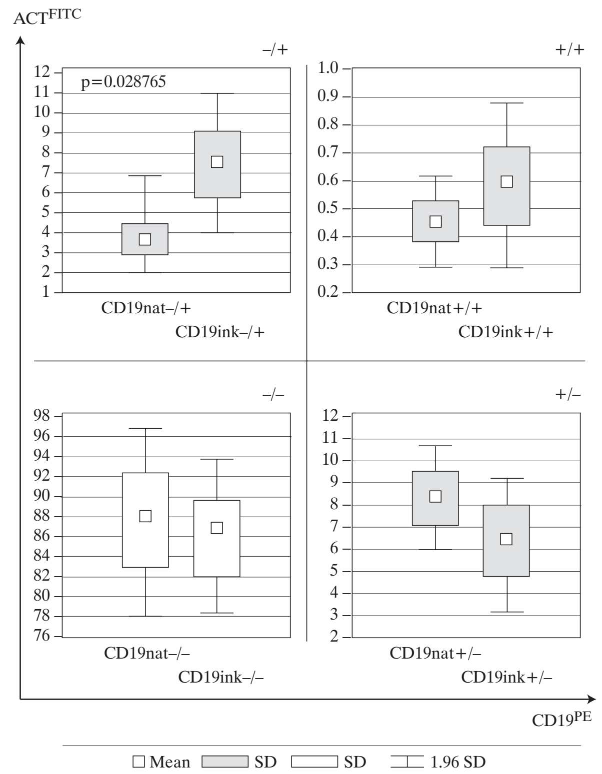


*Figure 2*. **Double staining (mean results for 9 experiments) with anti-ACT (FITC) and anti-CD19 (PE) – percentage of positive lymphocytes, native versus incubated with ACT preparation (point=mean, frame=SD, range=1.96 SD)**

indicate its link with innate immunity. Weaker reaction with B lymphocytes might also point to the regulatory role of ACT in the specific response. It has to be investigated further what is the link between ACT presence on the surface and activity of particular lymphocytes subpopulations and whether there is any relationship between ACT glycosylations profile and its influence on lymphocytes. Further investigation will be performed to establish the binding particularly with NK cells.

## Acknowledgement

Author wants to express gratitude to Mr. Artur Kostrzewa, MSc, for the excellent technical assistance with flow cytometry and to Professor Krzysztof Wiktorowicz for the support and discussion of results.

### References

1. Vila N, Obach V, Revilla M, Oliva R, Chamorro A. α1-Antichymotrypsin Gene Polymorphism in Patients With Stroke. Stroke, 2000; 31: 2103-5.
2. Gooptu B, Hazes B, Chang W-SW, Dafforn TR, Carrell RW, Read RJ, Lomas DA. Inactive conformation of the serpin a1-antichymotrypsin indicates two-stage insertion of the reactive loop: Implications for inhibitory function and conformational disease. PNAS, 2000; 97(1): 67–72.
3. Ikari Y, Mulvihill E, Schwartz SM. α1-Proteinase inhibitor, α1-Antichymotrypsin, and α2-Macroglobulin are the antiapoptotic factors of vascular smooth muscle cells. J Biol Chem, 2001; 276(15), 11798-803.
4. Sobieska M, Mikstacki A, Wiktorowicz K. Zmiany stężeń cytokin i wybranych białek ostrej fazy pod wpływem urazów wielonarządowych. Nowiny Lekarskie, 1998; 67(4): 515-23.
5. Weaver VM, Carson CE, Walker PR, Chaly N, Lach B, Raymond Y, Brown DL Sikorska M. Degradation of nuclear matrix and DNA cleavage in apoptotic thymocytes. J Cell Science, 1996; 109: 45-56.
6. Lanzrein AS, Johnston CM, Perry VH, Jobst KA, King EM, Smith AD. Longitudinal study of inflammatory factors in serum, cerebrospinal fluid, and brain tissue in Alzheimer disease: interleukin-1beta, interleukin-6, interleukin-1 receptor antagonist, tumor necrosis factor alpha, the soluble tumor necrosis factor receptors I and II, and alpha1-antichymotrypsin. Alzheimer Dis Assoc Disord, 1998; 12: 215-27.
7. Licastro F, Pedrini S, Caputo L, Annoni G, Davis LJ, Ferri C, Casadei V, Grimaldi LM. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? J Neuroimmunol, 2000; 103: 97-102.
8. Machyńska-Bućko Z, Sobieska M. Ilościowe zmiany wybranych białek ostrej fazy u chorych z ciężkim oparzeniem. Nowiny Lekarskie, 1998; 67(12): 1254-62.
9. Kaźmierczak M, Sobieska M, Wiktorowicz K, Wysoki H. Changes of acute phase proteins glycosylation profile as a possible prognostic marker in myocardial infarction. Int J Cardiol, 1995; 49: 201-7.
10. Kaźmierczak E, Sobieska M, Kaźmierczak M, Mrozikiewicz A, Wiktorowicz K. Intense acute phase response in ischemic patients. Int J Cardiol, 1999; 68: 69-73.
11. Kołowrotkiewicz A, Sobieska M, Wołowicka L, Mikstacki A. Estimation of acid glycoprotein (AGP) and antichymotrypsin (ACT) in patients with polytrauma. In: 7th European Congress on Intensive Care

Medicine, Innsbruck, 14-17 June 1994. Int Care Med, 1994; 20(Suppl. 2): S84.

12. Springer E, Sobieska M, Wiktorowicz K. Measurements of acute phase proteins in food allergy and tolerance. XVII International Congress of Allergology and Clinical Immunology, 15-20 October 2000, Sydney, Australia. Abstract P-540.

13. Laurell, CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Scand J Clin Invest, 1972; 124: 21-8.

14. Bøg-Hansen TC. Crossed immuno-affinoelectrophoresis: an analytical method to predict the result of affinity chromatography. Anal Biochem, 1973; 56: 480-3.

15. Zhanga S, Janciauskiene S. Multi-functional capability of proteins: α1-antichymotrypsin and the correlation with Alzheimer's disease. Journal of Alzheimer's Disease, 2002; 4: 115-22.

16. Robertson AS, Belorgey D, Lilley KS, Lomas DA, Gubb D, Dafforn TR. Characterization of the Necrotic Protein That Regulates the Toll-mediated Immune Response in Drosophila. J Biol Chem, 2003; 278(8): 6175-80.