Anti-DNA ("Cationic") and Ani-Histone ("Anionic") Auto-Antibodies: Two Sides of One Coin?

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Abstract: The mini review is focused on the analysis of published data and the results obtained by author with co-workers about biological activity of anti-DNA ("cationic") and anti-histone H1 ("anionic") auto-antibodies linked with autoimmunity and oncology. The feathures of anti-DNA and-anti histone auto- antibodies, which might be engaged in regulation of tumor cell progressing, are considered.

Keywords: Human blood serum, Auto-antibodies, Cytotoxicity, Pro-poliferative activity, Autoimmunity, Oncology.

INTRODUCTION

The cardinal feature of systemic autoimmune diseases is a humoral immune response targeted to intracellular proteins and nucleic acids. Auto antibodies (auto-Abs) possessing reactivity to different intracellular antigens which associate with systemic autoimmune diseases were also found in cancer patients [1-3]. The auto-Abs with reactivity to duble-stranded DNA and histones are a typical and frequently observed immunologic abnormality of autoimmune and tumor diseases [4, 5]. An analysis of the experimental data showed that these auto-Abs have an opposite effect on the tumor cells *in vitro*. Thus, Anti-DNA auto-Abs revealed cytotoxicity towards tumor cells [6-8], while the anti-histone auto-Abs posess a pro-proliferative activity towards the same cells *in vitro* [9, 10]. These data allow to assume that the balance of cationic and anionic antibodies can determine the human resistance or sensitivity to the tumor progressing.

A Cytotoxic Effect of Anti-DNA Auto Antibodies

It was found that anti-double stranded DNA IgGs purified from blood serum of patients with active systemic lupus erythematousus (SLE), using immobilized mammalian DNA are cytotoxic to different mammalian cell lines: L929, HL-60, Raji, K562, L929, MC [6-9]. It was shown, that death caused by anti-dsDNA IgG followed a process of apoptosis rather than necrosis. The treatment of the cells with cytotoxic anti-DNA autoantibodies induced inter nucleosomal DNA fragmentation and Annexin V binding to the cell surface characteristic of apoptotic pathway of cell death. [7]. The results suggest, that binding of anti-dsDNA IgG with MC membrane may activate endonuclease which will fracture the DNA and lead to programmed cell death. Using a immuno fluorescent staining, immunoblotting, radioimmunoprecipitation, and cell cycle analysis, have shown that anti-dsDNA IgG could bind to the membrane of rat mesangial cells (MC). It was shown, that the binding antigen is a 28 kDa protein, which disappeared, when the cells were treated in advance with proteinase K [8].

We also have shown that Anti-dsDNA sIgAs, which were obtained from human milk by sequential chromatography on protein A-sepharose, DEAE-fractogel and DNA-cellulose columns, possess toxic

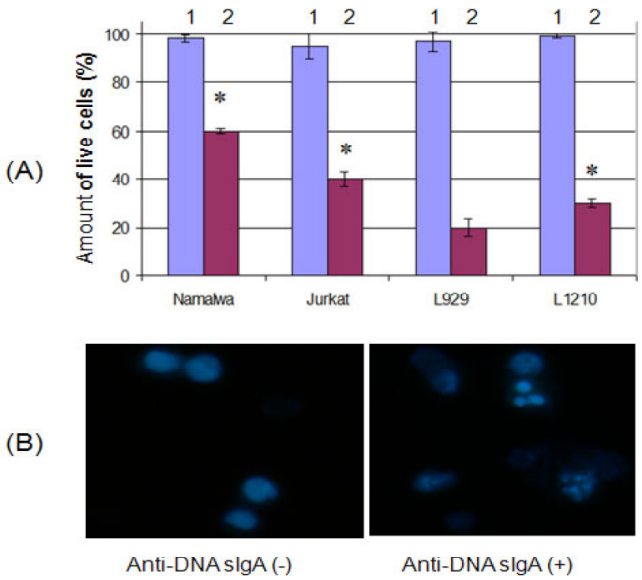

Figure 1: Influence of anti- DNA sIgA on the growth and survival of tumor and transformed cells *in vitro*. (**A**) - Cytotoxic activity of sIgA-antibodies. 1 - Control (number of cells in the absence of anti- DNA sIgA. 2 – amount of cells in the presence of anti- DNA sIgA. (*), P≤0.05. (**B**) - Condensation and fragmentation of chromatin of L1210 cells under the influence of anti- DNA sIgA.

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activity toward several transformed and tumor cells *in vitro* [11]. It was found, that the sensitivity of studied cells to Ab changed in orderL929> L1210> Jurkat> Namalva (Figure **1**). Annexin V – tests, as well as DNA-fragmentation tests showed that cell death under Abs action occurred by apoptosis.

These results demonstrate that the anti-DNA auto-Abs preparations, isolated from the blood serum of SLE patients and human colostrums are cytotoxic toward different tumor cells *in vitro*. The effect can be linked with their antigenic poly-specificity and/or DNAase activity [7, 11].

A Pro–Proliferative Effect of Anti-Histone Auto Antibodies

We have shown that blood serum of patients with multiple myeloma, similarly to blood serum of some patients with multiple sclerosis, and some colostrums of healthy mothers, contain Abs, capable of hydrolyzing calf thymus histone H1 [9, 12]. The specificity of Abs purification excludes their possible contamination by serum proteases. The affinity of catalytically active auto-Abs towards histone H1 allows us to classify them as anti-his H1 auto-antibodies. Our followed studies have shown that IgGs isolated by histone H1-Sepharose affinity chromatography from blood serum of the systemic lupus erythematosis, are capable of stimulating proliferation of human T-leukemia cells *in vitro* [9, 10]. At the same time, proliferation-stimulating activity was absent in IgGs purified from blood serum of healthy donors used as a control. Such activity was confirmed to be an intrinsic property of the IgG molecule, since it was preserved at gel filtration through HPLC column at strongly acidic pH. The cross-reactivity of anti-histone H1 IgGs towards membrane and cellular antigens of CEM T-cells was detected. These IgGs covalently coupled to biotin are capable of penetrating into CEM cells and were preferentially accumulated in the cytoplasm. This result indicats, that anti-histone H1 auto-Abs, isolated from the blood serum of SLE patients, are capable of stimulating a proliferation of human T-leukemia CEM cells. This effect can be linked with antigenic poly-specificity of these antibodies and/or their capacity to penetrate into the target cells (Figure **2**).

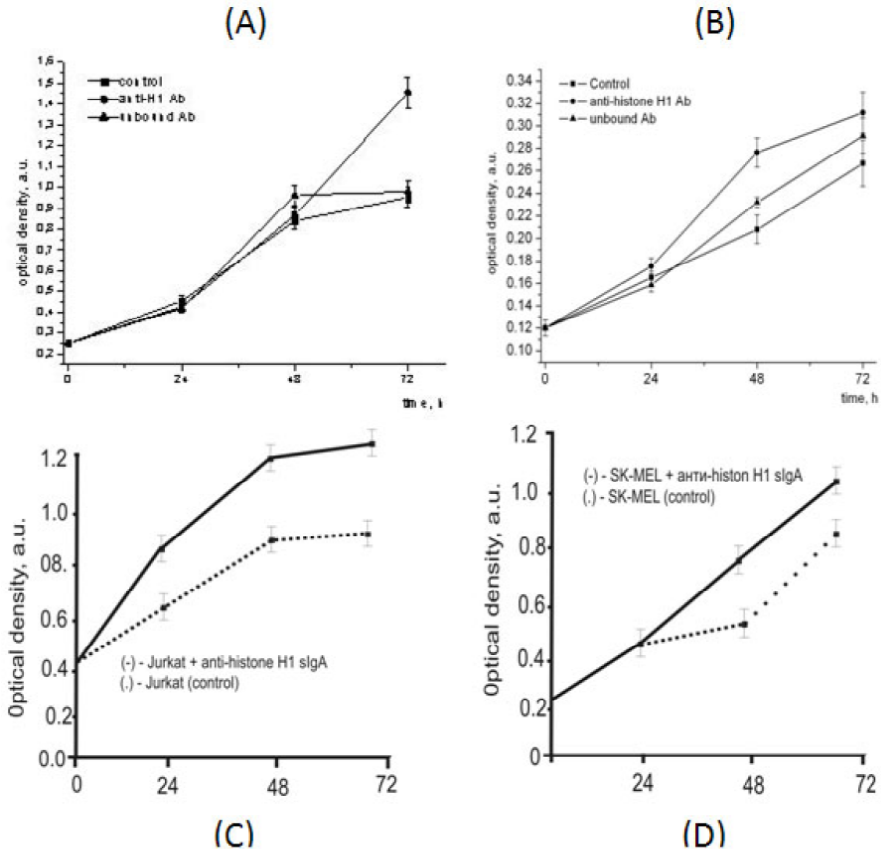

Figure 2: Influence of anti-histone H1 IgG (**A, B**) and anti-histone H1 sIgA (**C, D**) on growth and survival of a human T-leukemia CEM cells *in vitro*.

We also have shown, that electrophoretically homogeneous anti-histone H1 sIgAs obtained from human milk by sequential chromatography on protein A-Agarose, and histone H1-Sepharose respectively are able to stimulate proliferation of human T-leukemia Jurkat and human melanoma SK-MEL cells *in vitro* [13]. Mitogenic effect of these Abs was confirmed with an increase in different signal proteins, involved in cell proliferation (c-Myc, MAP-and cdc2-protein kinases), detected by Western-blot analysis. We also studied the antigenic reactivity of anti-histone H1 sIgAs towards SK-MEL cell proteins. It was observed, that these auto-Abs possess an affinity for a number of melanoma cell proteins with molecular masses of 60, 55, 48 and 38 kDa [13]. The cross reactivity of these auto-Abs (Figure **3**). could serve as an explanation of their mitotic activity toward the target cells.

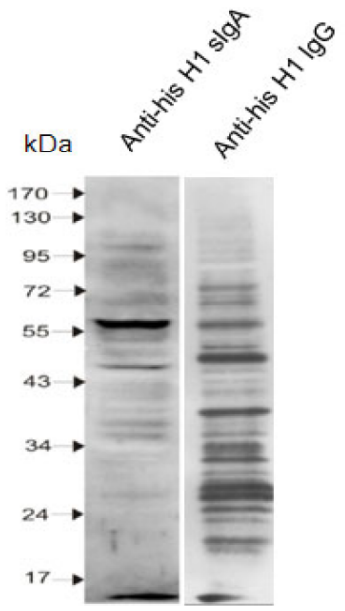

Figure 3: Western-blot analysis of cross-reactivity of biotinilated anti-histone H1 IgGs and anti-histone H1 sIgAs towards the CEM-cells Triton X-100 extracted cellular proteins.

Characterization of "Cationic" and "Anionic" Auto Antibodies

Since, antibodies possessing a cytotoxic , as well as , a pro-proliferative activities , were isolated by the affinity chromatography on the columns containing Sepharose beads bearing dsDNA or histone H1, they could be classified as anti - DNA or anti-histone H1 auto-Abs. In fact, origin of these antibodies remains unclear. Their appearance in a human blood serum could be linked with molecular mimicry of some self and foreign antigens. Hence, if the cytotoxic auto-Abs possess to bind negative charget double-stranded DNA, they could by named as "cationic" auto-Abs, otherwise, the pro-poliferetive active auto-Abs having affinity to positive charget histone H1 could be named as "anionic" auto-Abs. The short comparative characteristic of "cationic" and "anionic" auto-Abs demonstrated at Table **1**.

Obviously, that an important characteristic feature of these auto-Abs is their cross-reactivity to different cellular antigens, as well as, their catalytic activity towards nucleic acids and proteins. The obtained data outlined the correlation between cytotoxicity and DNA-hydrolyzing properties of a "cationic" auto-Abs [6-8], as well as mitogenic activity of an "anionic" auto-Abs and their proteolytic properties also have been demonstrated [9, 14-16]. Interestingly, that the level of proteolytically active IgGs in blood serum of SLE patients towards both histone H1 and myelin basic protein tightly correlates with the disease severity in these patients [17].

DISCUSSION

According to its antigenic specificity, the "cationic" and "anionic" auto-Abs could be also classified as antinuclear autoantibodies [18]. Circulating antinuclear autoantibodies typically found in autoimmune conditions, have also been detected in cancer patients

Table 1: The Characteristic of "Cationic" and "Anionic" Auto-Abs

"Cationic " auto antibodies	Purification on DNA-cellulose column	Cross-reactivity to cellular auto antigens	Nuclease activity	Cytotoxic effect toward tumor cells	Represented by IgA and IgG subclasses
"Anionic" auto antibodies	Purification on histone- H1 sepharose column	Cross-reactivity to cellular auto antigens	Protease activity	Pro-proliferative effect toward tumor cells	Represented by IgA and IgG subclasses

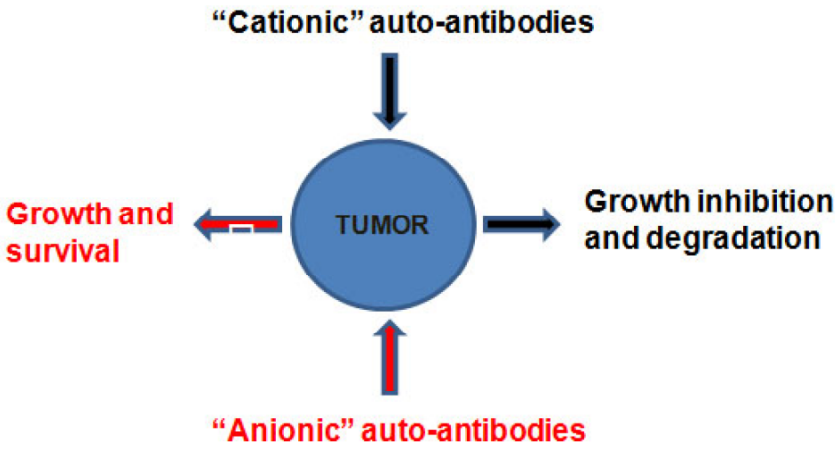

Figure 4: The balance between cytotoxic ("cationic"), and pro-proliferative ("anionic") auto-Abs influence on the human resistance and sensitivity to tumor progressing.

and in healthy older individuals [19]. It was found that antinuclear autoantibodies possess a complement-dependent and a complement independent toxicity towards different tumor cells [5, 6, 19]. There is a suggestion that antinuclear autoantibodies serve as anti neoplastic immune-surveillance agents [20]. On the other hand, there are data demonstrating, that some antinuclear auto-Abs possible engaged in stimulation of a development malignancy [9, 10]. Hence, it is possible that the balance between the cytotoxic and the pro-proliferative auto-Abs in human blood serum could serve as a positive or a negative factors for tumor progression (Figure **4**).

CONCLUSION

Summarising data shows, that cross - reactive "cationic" and "anionic" auto-Abs have opposite biologic activity. They could be present in healthy human as well as in patients with diseases. The balance between "cationic" and "anionic" auto-Abs could serve as one as the important factors, determining the personal immune status (so-colled "immunculus" [21]), which can determine of the human sensitivity or resistancy towards some autoimmune and oncological diseases.

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ABBREVIATION

- Abs antibodies
- Auto-Abs auto antibodies
- SLE systemic lupus erythematousus

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