EXPERIMENTAL WORKS

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THE INTERACTION BETWEEN POLYREACTIVE IMMUNOGLOBULINS AND ANTIGENS IS COMPLETELY NONSPECIFIC

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The interaction of PRIGs with various antigens differs radically from the interaction of specific antibodies and the corresponding antigen. First of all, this difference lies in the lower specificity of PRIGs, although there are other fundamental differences. For example, the binding of PRIGs and antigen is extremely dependent on the temperature at which the process occurs, whereas the binding of specific antibodies to antigen is not very dependent on temperature. There are also a number of substances, such as Tween 20, that significantly affect the binding of PRIGs to antigens, but have a much weaker effect on the binding of specific antibodies. This article is devoted to studying the degree of non-specificity of the PRIGs and antigen reaction, and the ability of serologically unrelated antigens to inhibit this interaction. According to the data presented in this article, the interaction between PRIGs and antigens is completely nonspecific.

K e y w o r d s: antigen-antibody interaction, PRIGs-antigens interaction, inhibition of interaction, monoclonal antibodies.

One of the main characteristics of antibodies is their high specificity of binding to corresponding antigens and lack of interaction with other antigens. In other words, an antibody should, ideally, bind only to the antigen that induced the synthesis of that antibody. However, in practice, antibodies can often bind, albeit much more weakly, to other, so-called related antigens, and in some cases, as has become known in recent years, even to unrelated antigens.

The problem of the body producing antibodies with high specificity to countless antigens and the mechanism of interaction between antibodies and antigens has been studied for many decades [1] and is now well understood. Basically, the specificity of antibodies is related to the fact that their structures are mutually complementary to the corresponding antigens and fit together like a “lock and key” [2]. In addition, the electrical charges of the interacting regions of the antibody and antigen are usually opposite, i.e., a negative charge corresponds to a positive charge and vice versa. In cases where hydrophobic interaction contributes significantly to antigen-antibody interaction, these sites are also located opposite to each other on the interacting surfaces of the antigen and antibody.

Later, after the discovery of so-called natural antibodies [3-8], the problem of their interaction with antigens was largely solved. It became known that natural antibodies, which are produced by organisms of mouse, rabbit, human, ets., in the absence of stimulation by the corresponding antigen, are generally less specific than classical antibodies produced by the body after primary and especially after repeated immunization. In addition, the bond between natural antibodies and antigens is much weaker than that between highly specific antibodies and corresponding antigens.

Several decades ago, we discovered [9-10] that human and animal sera, after undergoing certain treatments, acquired the ability to bind to a variety of serologically unrelated antigens. Thus, the

question arose about the mechanism of this type of interaction. Later, in our experiments, it was shown that not only serum immunoglobulins but also highly specific antibodies can, under certain conditions (a sharp shift in pH, high concentrations of chaotropic ions, exposure to active forms of oxygen, etc.), lose their specificity and acquired the ability to bind to any antigen. We called these modified antibodies as polyreactive immunoglobulins (PRIG), and later we found that intact human and animal sera contain a significant amount of immunoglobulins with PRIG properties [11].

In connection with the above, the question arises about the mechanism of interaction between PRIG and antigens and the degree of specificity (or non-specificity) of this interaction. This article presents the results of our studies on the influence of the conditions of transformation of highly specific monoclonal antibodies into PRIG, as well as the results of studies on the specificity of the binding process of such PRIG with various serologically unrelated antigens.

Materials and Methods

In this study, we used bovine serum albumin (BSA), rabbit serum albumin (RSA), human serum albumin (HSA), chicken egg albumin (ovalbumin or OVA), and horse myoglobin (HM) as antigens. All these reagents were commercial products from Sigma, USA. As specific antibodies we used monoclonal antibodies (mAb) specific to ovalbumin (anti-OVA) or BSA (anti-BSA), which were also products of Sigma, USA.

In order to obtain PRIGs that are not a heterogeneous product of numerous antibody-producing clones, but rather a relatively homogeneous reagent, we used instead of serum immunoglobulins, the commercial mAbs (anti-OVA or anti-BSA) that were transformed into PRIGs as follows. To do this, 5 µl of mAbs was mixed with 5 µl of KSCN solution of a certain molarity, the mixture was incubated for 5 min at 22-25°C, and then diluted 100 times by adding 1 ml of buffered physiological NaCl solution (BPS) to the mixture. As it was shown in our preliminary experiments, the preparations obtained in this way contained a portion of intact mAbs and a portion of immunoglobulin molecules transformed into nonspecific PRIGs.

To determine the amount of specific antibodies or PRIGs that were bound to the antigens immobilized on immunological plates we used the enzyme-linked immunosorbent assay (ELISA). Antigens were coupled to 96-cell NUNC immunological plates by incubation of their solution (5 mg/ml) in 1% of NH_4HCO_3 + 0.01%NaN_3 at 4°C during 20-24 h. Then, directly before using these plates, the solution with unbound antigen was removed and the plates were washed three times with phosphate buffered (pH 7.2) physiological solution NaCl (PBS) + 0.1% Twin 20 (TBS).

0.1 ml of the studied solution of mAbs or PRIG were put into wells of plates covered with certain antigens and the plates were incubated during 1 hour either at room temperature (for studying mAbs binding) or at 37°C (for studying PRIG binding), then the plates were thoroughly washed with TBS and the amount of mAbs or PRIG bound to immobilized antigen was determined. To do this the plates were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (Sigma, USA) during 60 min at 4°C. Then the unbound conjugates were carefully washed away, and peroxidase substrate (the solution of orthophenilendiamine (1 mg/ml) in 0.01 M phosphate buffer, pH 5.0 and 0.003% H_2O_2) was added. After color development the reaction was stopped by adding 50 µl of 2 M sulfuric acid per well, and optical density was measured by microphotocolorimeter ELx800 (BIO-TEK) at 490 nm.

Results and Discussion

Fig. 1 shows the results of studying the interaction of specific mAbs (anti-BSA and anti-OVA) with corresponding or unrelated antigens coupled to microplates. As expected, highly specific mAbs bound well to the corresponding antigen and showed virtually no reaction with unrelated antigens. Only at the highest antibody concentrations (commercial sample dilution 1:50,000) a slight binding of these mAbs to unrelated antigens was observed, and further dilution by a factor of 2, 4, and 8 resulted in almost complete absence of binding. At the same dilutions, the binding of mAbs to the corresponding antigen was pronounced and directly proportional to the antibody concentration. This indicates the high specificity of mAbs, and their extremely weak binding to unrelated antigens could be explained by the presence of very low amounts of PRIGs admixture in the antibody samples studied.

On the other hand, if solutions of various antigens (at different concentrations) were mixed with solutions of each of these mAbs, only the corresponding antigen blocked the binding of the antibody

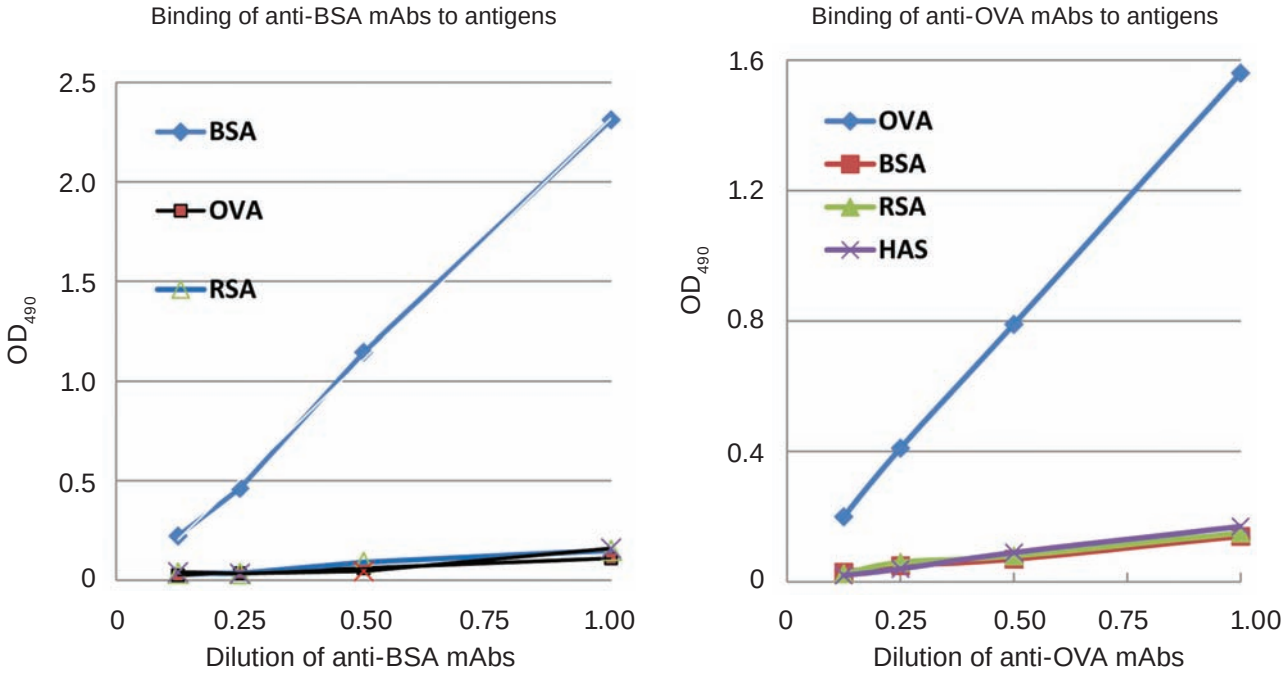

Fig. 1. Binding of anti-BSA or anti-OVA mAbs to different antigens immobilized on microplates

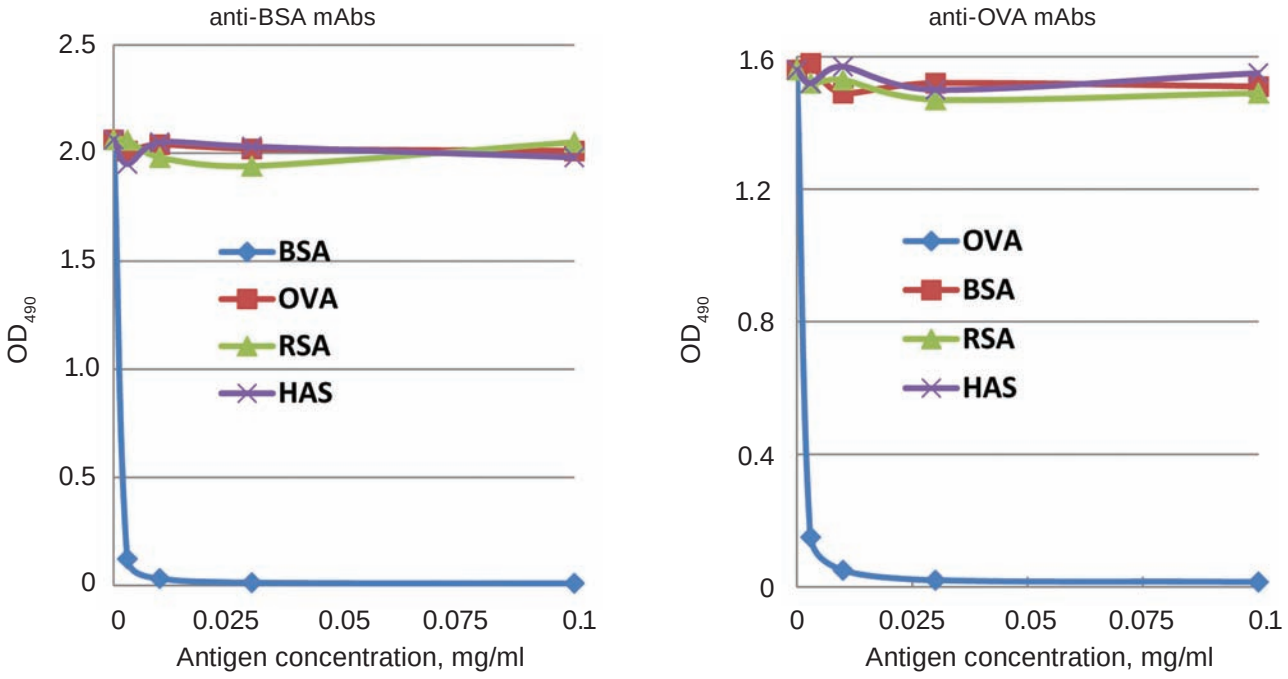

Fig. 2. Blocking of anti-BSA or anti-OVA mAbs only by corresponding antigens and no blocking by serologically nonrelated antigens

to the same antigen that was coupled to the microplates. The other unrelated antigens had virtually no effect on this process (Fig. 2). Thus, the mAbs that we used were highly specific, i.e., they could bind only to the corresponding antigen that was either in a solution or coupled to the microplates. Unrelated antigens had virtually no effect on the interaction of mAbs with the corresponding antigen.

A completely different situation is observed when these highly specific mAbs are transformed

into non-specific PRIGs under the influence of high concentrations (from 2.0 to 6.0 M) of chaotropic ions, in this case potassium thiocyanate, KSCN. Fig. 3 shows how the concentration of KSCN affects the ability of anti-BSA mAbs to bind nonspecifically to one of the unrelated antigens, namely ovalbumin or rabbit albumin. It can be seen that with an increase in KSCN concentration to 2.0-4.0 M, the activity of the obtained PRIGs increases to a certain level. A further increase in KSCN concentration leads to a decrease in PRIGs activity, which is apparently due to the destruction of some of the formed PRIGs under the influence of 5.0-6.0 M KSCN.

We observed a similar relationship (not shown) as a result of the transformation of anti-OVA mAbs into PRIGs and their subsequent binding to unrelated antigens, namely BSA or RSA. For this reason, in the further course of this work, we induced the transformation of specific antibodies into PRIGs by incubating the specified mAbs with a 3.0 M KSCN solution for 5 min at 25°C.

Fig. 4 shows the interaction between the PRIGs obtained in this way and serologically unrelated antigens coupled to the plates. As can be seen from the figure, the PRIGs we obtained acquired the ability to bind, albeit slightly weaker than the initial mAbs with the corresponding antigen, but with a wide variety of antigens, and, very importantly, with approximately the same efficiency. Thus, the previously highly specific mAbs (anti-BSA and anti-OVA) under the influence of 3.0 M KSCN solution became nonspecific, or in other words, they acquire polyreactive properties.

This raises the question: why are PRIGs able to bind to unrelated antigens? Is it because the PRIGs population contains some immunoglobulins that bind to one antigen, some that bind to another, and so on, or are PRIGs molecules homogeneous and each molecule capable of binding to any of the unrelated antigens? The following experiment provides an answer to this question. In this experiment, PRIG samples were pre-incubated with different antigens (to partially block PRIGs with these antigens) and then the remaining PRIG activity was evaluated in relation to various antigens covered on microplates (Fig. 5).

The data presented in Fig. 5 regarding PRIGs obtained during the transformation of anti-OVA mAb using 3.0 M KSCN allow us to conclude that PRIGs is completely non-specific with regard to the antigens we used. Similar data (not shown) were also obtained by us if we used PRIGs, that were obtained by transformation of anti-BSA mAbs by treatment them with a 3.0 M KSCN solution. These data indicate that the obtained PRIG preparations bind completely nonspecifically to unrelated antigens not because they contain a small fraction of molecules specific to each nonrelative antigens. On the contrary, PRIG preparations are apparently relatively homogeneous, and each PRIG molecule has the ability to bind to any of the studied antigens.

It should also be noted that earlier we have shown [11] that in the presence of certain antigens,

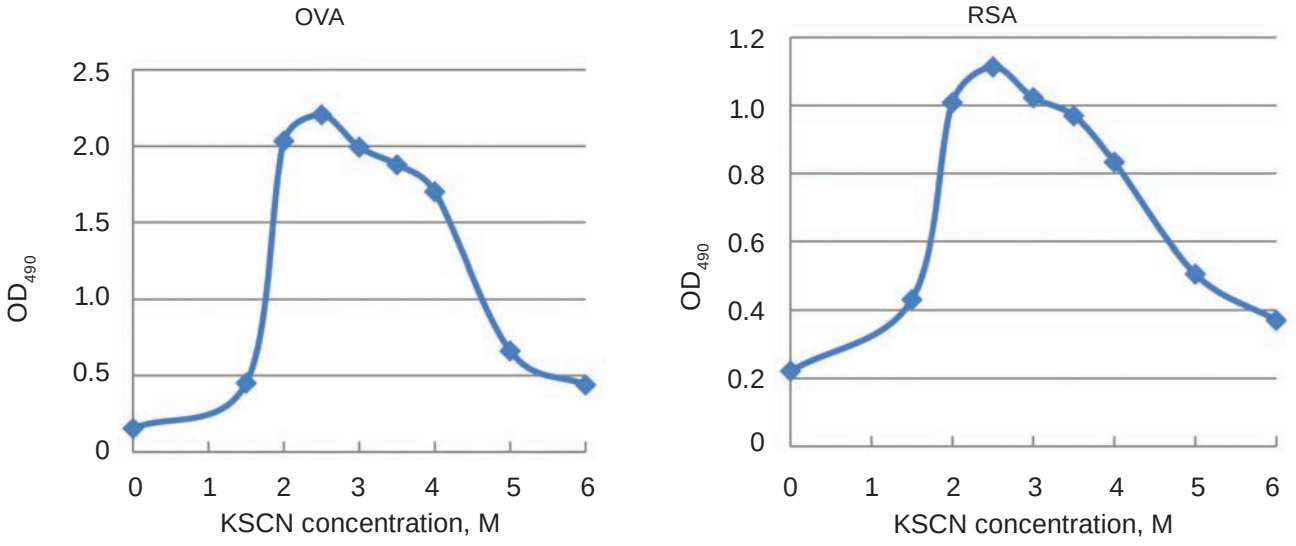

Fig. 3. Effect of KSCN concentration on transformation of anti-BSA mAbs into PRIGs and their reactivity with OVA or RSA coupled to microplates

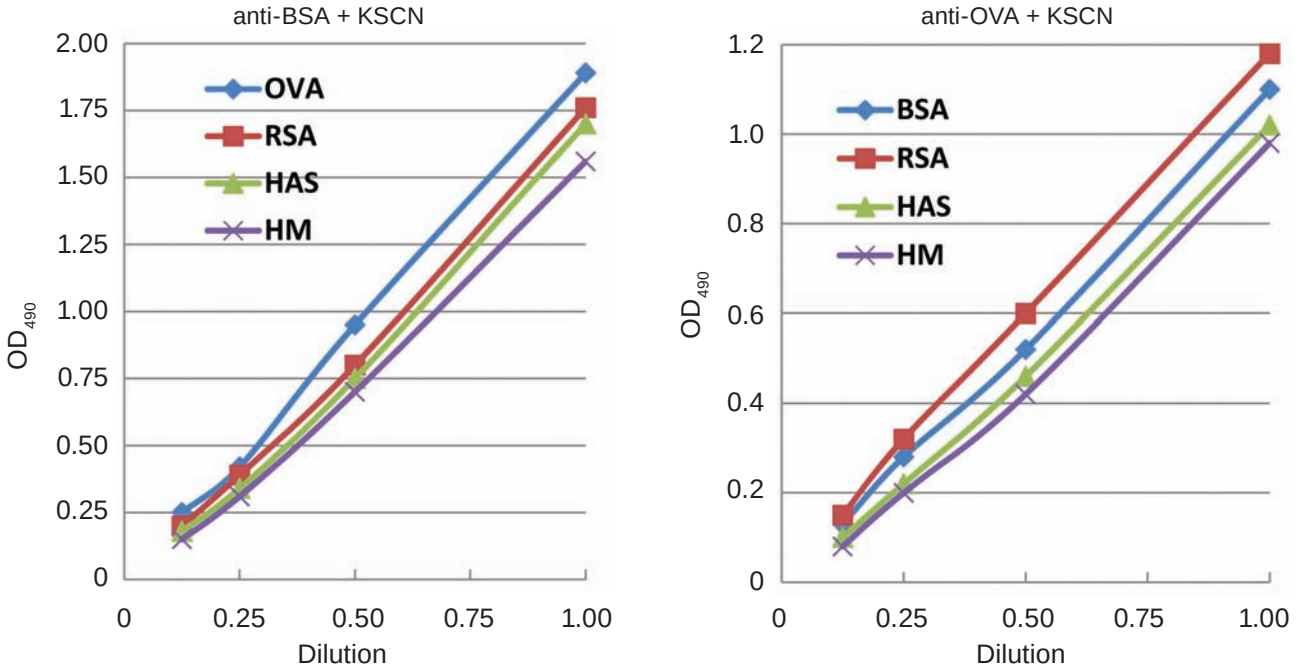

Fig. 4. Nonspecific binding of PRIGs, obtained by transformation either anti-BSA or anti-OVA mAbs with 3.0 M KSCN. to nonrelated antigens (OVA, RSA, HAS, HM or BSA) that were coupled to microplates

PRIGs were not only not inhibiting by them, but even became more reactive towards various antigens. Such antigens, which do not weaken but enhance the activity of PRIGs, include horse cytochrome C, protamine, and chicken egg lysozyme. The mechanism of this enhancement of PRIG reactivity is not yet fully understood, although it can be assumed that in the presence of these antigens, more favorable conditions are created for the appearance of hydrophobic sites on the surface of PRIG molecules (due to the dynamics of polypeptide chains) [11], which are necessary for the binding of PRIG to antigens.

The fact that hydrophobic interaction is the main mechanism for binding PRIGs to antigens is evidenced by experiments in which substances that suppress hydrophobic interaction weaken PRIG-antigen interaction, although the same substances have almost no effect on the binding of specific antibodies to the corresponding antigens. Earlier we have shown [12, 13] that Tween 20 and ANS, which have an affinity for hydrophobic regions of molecules, significantly reduce the ability of PRIGs to bind to antigens coupled to plates. We have also previously shown that PRIGs interact more effectively with protein antigens if the latter have been pre-denatured by heating [12, 13].

Thus, the data we obtained allow us not only to identify the fact of nonspecific interaction between PRIG and antigens, but also to draw conclusions about the cause of this nonspecificity. Unlike specific antibodies, where the interacting surfaces of the antigen and antibody are mutually complementary and fit together like a key and a lock, such complementarity is not necessary for the interaction of PRIGs and antigens. It is sufficient that, due to the molecular dynamics of polypeptide chains, hydrophobic regions appear on the surface of interacting molecules for a short time, and then, when these immunoglobulin molecules collide with the hydrophobic regions of antigens, a fairly strong bond can form.

As we established earlier [14], the strength of the non-specific bond between PRIGs and the antigen is quite high, since it cannot be broken by incubation PRIG-antigen complex with an excess of antigen (as is possible to do in the case of specific high affinity antibodies). To break the bond formed between PRIGs and the antigen, higher concentrations of chaotropic ions, such as KSCN, or higher (lower) pH values of the solution are required than those necessary to break the bond between specific antibodies and the antigen.

The data we have obtained also allow us to conclude that the ability of PRIGs to bind nonspecifically to various antigens, including autoantigens, may not only have a positive effect (as in the opsonization of microbial antigens) but may also induce

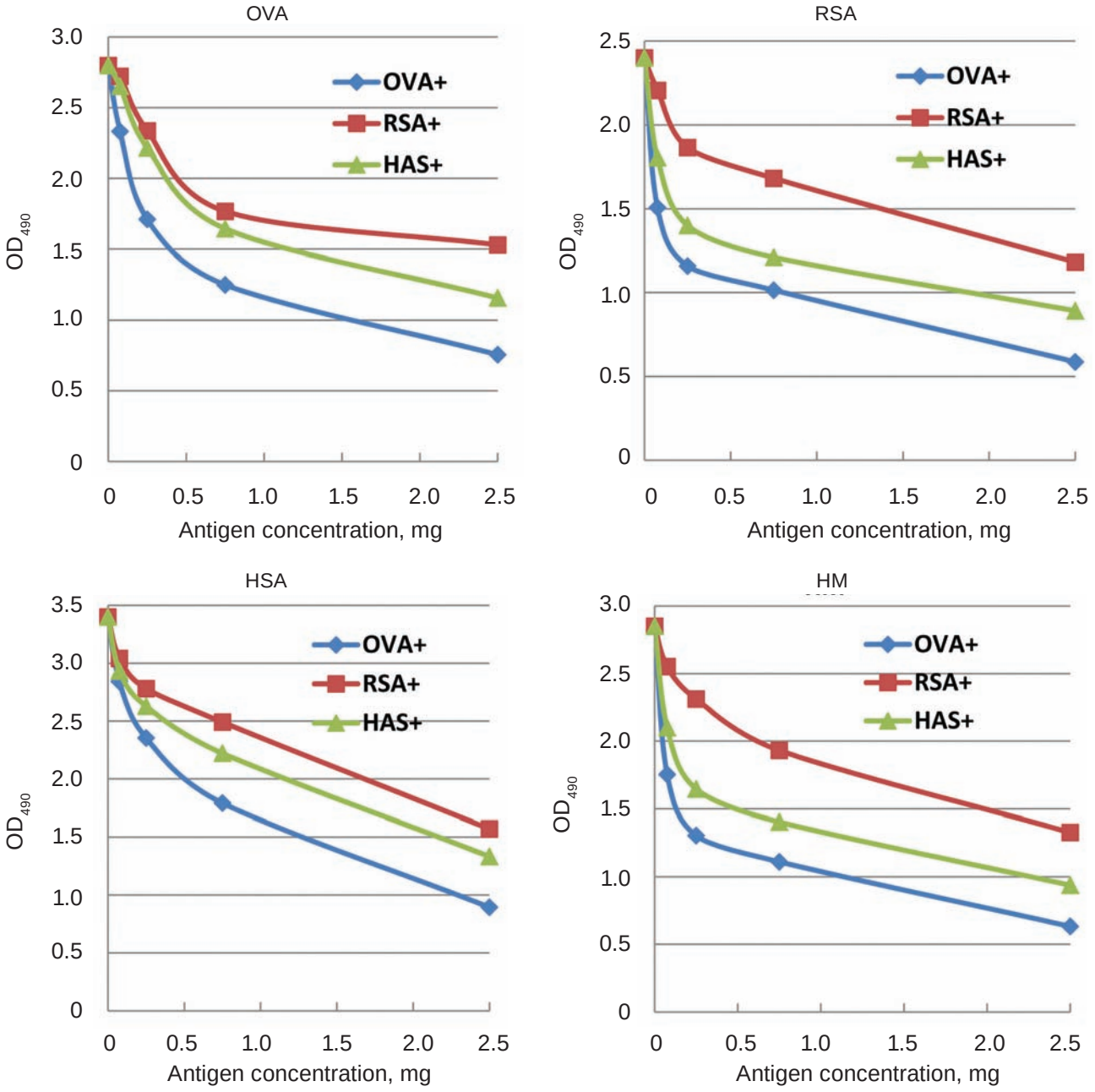

Fig. 5. Non-specific blocking of PRIGs by various antigens (OVA, RSA, and HSA) at antigen concentration 0.5-2.5 mg/ml. PRIGs were obtained by transformation of anti-BSA antibodies with 3.0 M KSCN treatment (5 min at 25°C) and titrate on the plates covered with OVA, RSA, HSA and HM

the gradual development of certain pathological processes such as atherosclerosis or other diseases associated with the development of autoimmune reactions. The fact that natural antibodies can react with autoantigens was also established by others [15-17]. In this regard, further study of the properties of PRIGs and their biological effects appears to be highly relevant.

Conclusions.

1. Specific monoclonal antibodies can be transformed into nonspecific polyreactive immunoglobulins using 3.0-5.0 M KSCN.

2. Polyreactive immunoglobulins are not only capable of binding to a variety of serologically unrelated antigens, but their inhibition can also be achieved by a variety of antigens.

3. The degree of inhibition of polyreactive immunoglobulins in terms of their ability to bind to a certain antigen X using this antigen is no higher than that of other serologically unrelated antigens Y, Z, etc.

3. The data we obtained indicate that the reaction between polyreactive immunoglobulins and antigens is completely nonspecific.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВЗАЄМОДІЯ МІЖ ПОЛІРЕАКТИВНИМИ ІМУНОГЛОБУЛІНАМИ ТА АНТИГЕНАМИ Є АБСОЛЮТНО НЕСПЕЦИФІЧНОЮ

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Взаємодія ПРІГ із різноманітними антигенами кардинально відрізняється від взаємодії специфічних антитіл і відповідними антигенами. Перш за все ця відмінність полягає у нижчій специфічності ПРІГ, хоча існують і інші фундаментальні відмінності. Зокрема, зв'язування ПРІГ і антигену надзвичайно сильно залежить від температури, за якої відбувається цей процес, тоді як зв'язування специфічних антитіл з антигеном є значно менш температурозалежним. Існує також ряд речовин, наприклад Твін 20, які помітно впливають на зв'язування ПРІГ з антигенами, але значно слабше – на зв'язування специфічних антитіл. Ця стаття присвячена вивченню ступеня неспецифічності реакції ПРІГ і антигенів, здатності серологічно неспоріднених антигенів інгібувати цю взаємодію. Відповідно до отриманих даних, взаємодії ПРІГ з антигенами є повністю неспецтфічною.

Ключові слова: взаємодія антиген-антитіло, взаємодія PRIG-антигенів, інгібування взаємодії, моноклональні антитіла.

References

1. Berzofsky JA, Berkover AJ. Antibody-antigen interaction. Fundamental Immunology. Ed. by Paul WE., 1989, Raven Press, New York, P. 3-88.
2. Landsteiner K. The specificity of serological reactions. Rev. edn. Harvard University Press, Cambridge, 1945. 310 p.
3. Avrameas S, Dighiero G, Lymberi P, Guilbert B. Studies on natural antibodies and autoantibodies. *Ann Immunol (Paris).* 1983; 134D(1): 103-113.
4. Avrameas S. Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. *Immunol Today.* 1991; 12(5): 154-159.
5. Avrameas S, Ternynck T. The natural autoantibodies system: between hypotheses and facts. *Mol Immunol*. 1993; 30(12): 1133-1142.
6. Notkins AL. Polyreactivity of antibody molecules. *Trends Immunol*. 2004; 25(4): 174-179.
7. Zhou ZH, Tzioufas AG, Notkins AL. Properties and function of polyreactive antibodies and polyreactive antigen-binding B cells. *J Autoimmun*. 2007; 29(4): 219-228.
8. Dimitrov JD, Planchais C, Roumenina LT, Vassilev TL, Kaveri SV, Lacroix-Desmazes S. Antibody polyreactivity in health and disease: statu variabilis. J Immunol. 2013;191(3):993-999.
9. Bobrovnik SA. Activation of "silent" antibodies and their interaction with antigens. *Ukr Biokhim Zhurn*. 1990; 62(5): 86-89. (In Russian).
10. Bobrovnik SA. Polyreactive immunoglobulins: molecular properties and functions. *Comments Mol Cell Biophys.* 1999; 9: 323-356.
11. Bobrovnik SA, Demchenko MO, Komisarenko SV. Interaction peculiarities of polyreactive immunoglobulins and various antigens. *Ukr Biochem J*. 2014; 86(1): 68-74. (In Russian).
12. Bobrovnik SA. Polyreactive immunoglobulins recognize hydrophobic parts of proteins. *Ukr Biokhim Zhurn*. 2001; 73(2): 116-122. (In Russian).
13. Bobrovnik SA. Mechanisms of interaction of polyreactive immunoglobulins and protein antigens. *Ukr Biokhim Zhurn*. 2002; 74(2): 37-44. (In Russian).
14. Bobrovnik SA. Avidity of polyreactive immunoglobulins. *Ukr Biochem J*. 2014; 86(6): 183-189. (In Russian).
15. Avrameas S, Guilbert B, Dighiero G. Natural antibodies against tubulin, actin myoglobin,

thyroglobulin, fetuin, albumin and transferrin are present in normal human sera, and monoclonal immunoglobulins from multiple myeloma and Waldenström's macroglobulinemia may express similar antibody specificities. *Ann Immunol (Paris).* 1981; 132C(2): 231-236.

16. Guilbert B, Dighiero G, Avrameas S. Naturally occurring antibodies against nine common antigens in human sera. I. Detection, isolation and characterization. *J Immunol.* 1982; 128(6): 2779-2787.

17. Dighiero G, Guilbert B, Avrameas S. Naturally occurring antibodies against nine common antigens in humans sera. II. High incidence of monoclonal Ig exhibiting antibody activity against actin and tubulin and sharing antibody specificities with natural antibodies. *J Immunol.* 1982; 128(6): 2788-2792.