

# КОРОТКІ ПОВІДОМЛЕННЯ

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## AGE CHANGES OF HUMAN SERUM POLYREACTIVE IMMUNOGLOBULINS (PRIG) ACTIVITY

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*It has been determined that activity of serum polyreactive immunoglobulins (PRIG) changes with age in practically healthy people at the age from twenty five to seventy. Therewith, the activity of serum IgG PRIG increases most of all (about 3-4 times), while IgM PRIG activity, on the contrary, does not increase, but sometimes even decreases with age. IgA PRIG activity varies significantly more than IgG PRIG activity and, besides, IgA PRIG significantly less depends on age than IgG PRIG. The age changes in the activity of human serum PRIG, belonging to different types of immunoglobulins, may evidence for the important functional role of these immunoglobulins that has to be clarified.*

*Key words: activity of polyreactive IgG, age changes, ELISA.*

It is known that blood serum immunoglobulins in humans and animals play important role in their protection from different infections, because they act as antibodies able to specifically bind to corresponding antigens, neutralizing toxins or opsonizing antigens of microorganisms thus promoting their phagocytosis by special cells, phagocytes. Significant deviations of serum immunoglobulins concentration from norm, as a rule, is the evidence for serious diseases [1–4].

Blood serum immunoglobulins of healthy humans and animals, which are antibodies against different antigens, may be divided into so-called natural antibodies (the antibodies, synthesized without visible organism's immunization), and classic antibodies, which are produced by organism in response to its active immunization by specific antigens or to infection. Both antibodies are specific for corresponding antigens, though it is known that natural antibodies usually possess less specificity and less affinity in comparison to the antibodies, obtained as a result of several artificial immunizations.

In addition to the above-mentioned two classes of antibodies, which are specific for a certain antigen and are well studied and described in numerous immunology manuals, we have also detected the so called polyreactive immunoglobulins (PRIG) [5–7].

These PRIG are able to bind nonspecifically to various antigens, including antigens of their own organism. It has been also determined that PRIG interact nonspecifically with antigens by different mechanism in comparison with well studied specific antibodies. [8–10].

Later, our data on PRIG existence were also confirmed by other researchers [11–13]. Further study of PRIG biological properties has demonstrated that they significantly differ from those of classic antibodies in terms of the way of interaction with antigens [7, 10], and possibly in terms of their biological functions [8]. Thus, the investigation of biological role of PRIG, under the change of organism's immune status or development of different pathologies, is very important. The investigation of PRIG concentration (or PRIG activity) not only under the conditions of different diseases, but also in the process of organism's aging is also of specific interest. This investigation is devoted to the study of the above problem.

### Materials and Methods

Human serum albumin (HSA) produced by ICN Biomedical Inc., (USA) was used in this work as an antigen. As it was demonstrated earlier, just this antigen is preferable for detecting human serum

PRIG. Goat antibodies conjugated with horse-radish peroxidase specific for mice IgG (Sigma, USA), were used as secondary antibodies in ELISA reaction. Ortho-phenylene diamine (Sigma, USA) and also hydrogen peroxide (Ternopharm, Ukraine) were used as substrate for immunoferrment reaction. Sera of people aged about 25 and more than 70 were obtained by the standard method.

Sorption of HSA on immunology plates was done with the help of the authors' method of drying the solution HSA (3.0-5.0  $\mu\text{g/ml}$ ) in 1% solution of  $\text{NH}_4\text{HCO}_3$  (pH 9.0) at 37 °C [13]. It was demonstrated earlier that such antigen sorption is the most suitable for PRIG detection. Serum samples of the examined people, dissolved many times (no less than 300-1000 times) in the physiologic solution NaCl, buffered by phosphate buffer (pH 7.2-7.4) and containing 0.05% twin 20 and also 0.1 mg/ml of protamin, were put into the well of immunological microplates with fixed HSA. The plates with the mentioned serum samples were incubated for an hour at 37 °C, and then they were carefully washed with running water from not-bound PRIG; after that goat anti-IgG, anti-IgA or anti-IgM antibodies, conjugated with horse-radish peroxidase, were added.

Then the plates were incubated with the above-mentioned conjugate during one hour at 4 °C; after that they were carefully washed up, and substrate for horse radish peroxidase, namely the mixture of ortho-phenylene diamine (1 mg/ml) and 0.003%  $\text{H}_2\text{O}_2$ , were added. After the development of substrate color, the reaction was stopped by adding 0.06 ml of 2 M sulphuric acid to each well, and the color of plate well was measured by microphotocolorimeter ELx800 BIO-TEK, at wave length of 490 nm.

### Results and Discussion

To have an ability to evaluate PRIG activity in human and animal sera, it is necessary to be able to distinguish between immunoglobulins belonging to PRIG and those which are natural antibodies with low affinity and are able to bind specifically to corresponding antigens. The method allowing this was proposed in one of our latest works. It is based on the idea that serum albumin of the animal, whose PRIG is supposed to be determined, should be used as an antigen for detecting PRIG, because, as a rule, the serum does not contain natural antibodies to its own serum albumin. The other important condition of PRIG determination is the use of special conditions

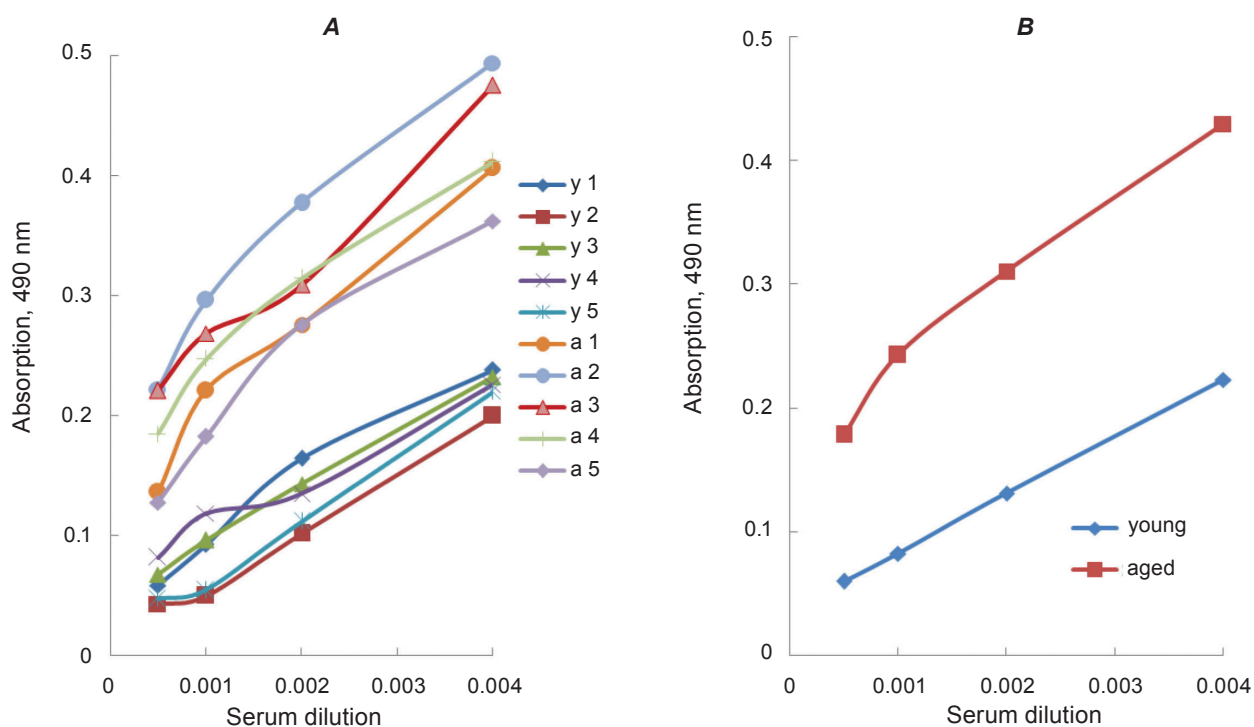


Fig. 1. Titration curves of five serums of young (y) and five serums of aged (a) people (A) for content of IgG PRIG, as well as average values of obtained magnitudes (B)

under which PRIG activity significantly increases, as that was shown earlier [15].

Curves of IgG PRIG titration in serums of ten practically healthy young or aged people are presented on Fig. 1. Age of five investigated people was up to 25 years, but the other five ones were about 70 years old. As that appears from Fig. 1, activity of serum IgG PRIG of aged people is higher in general than this indicator in young adult people. If to calculate the average levels of serum IgG PRIG activity in these two groups of people, titers in aged people are approximately 3-4 times higher than in young ones. According to literature data [2-4], the concentration of IgG immunoglobulins may increase with age, but significantly less than the activity of serum IgG PRIG determined by us. The obtained data show that specific activity of IgG PRIG per unit of concentration of serum immunoglobulins also increases with age.

In contrast to the increase of titers of serum IgG PRIG, the activity of IgM PRIG in serums of the same individuals in our tests did not increase with age, but significantly more varied by size; besides, it was even lower in aged people than in some young

ones (Fig. 2). Therewith we may conclude that the patterns detected by us in respect of IgG PRIG are not observed in respect of serum IgM PRIG. They rather depend on individual specificities of the immune system of the given object.

Almost the same conclusions may be made regarding the change in activity of serum IgA PRIG with age (Fig. 3). Like the activity of serum IgM PRIG, which is presumably proportional to concentration of these molecules in serums, the activity of serum IgA PRIG varies significantly more as compared to activity of serum IgG PRIG. At the same time, the activity of serum IgA PRIG in aged people insignificantly increases, though not so obviously as activity of serum IgG PRIG.

Thus, the data obtained prove that serum PRIG activity may change with age towards the increase; besides, the most obvious changes are observed regarding IgG PRIG activity. It increases 3-4 times with the increase of individuals' age from 25 to 70 years. The activity of serum IgM and IgA PRIG varies significantly more than the IgG PRIG activity, and the increase of their activity is not so obvious. The changes in activity of serum PRIG of people,

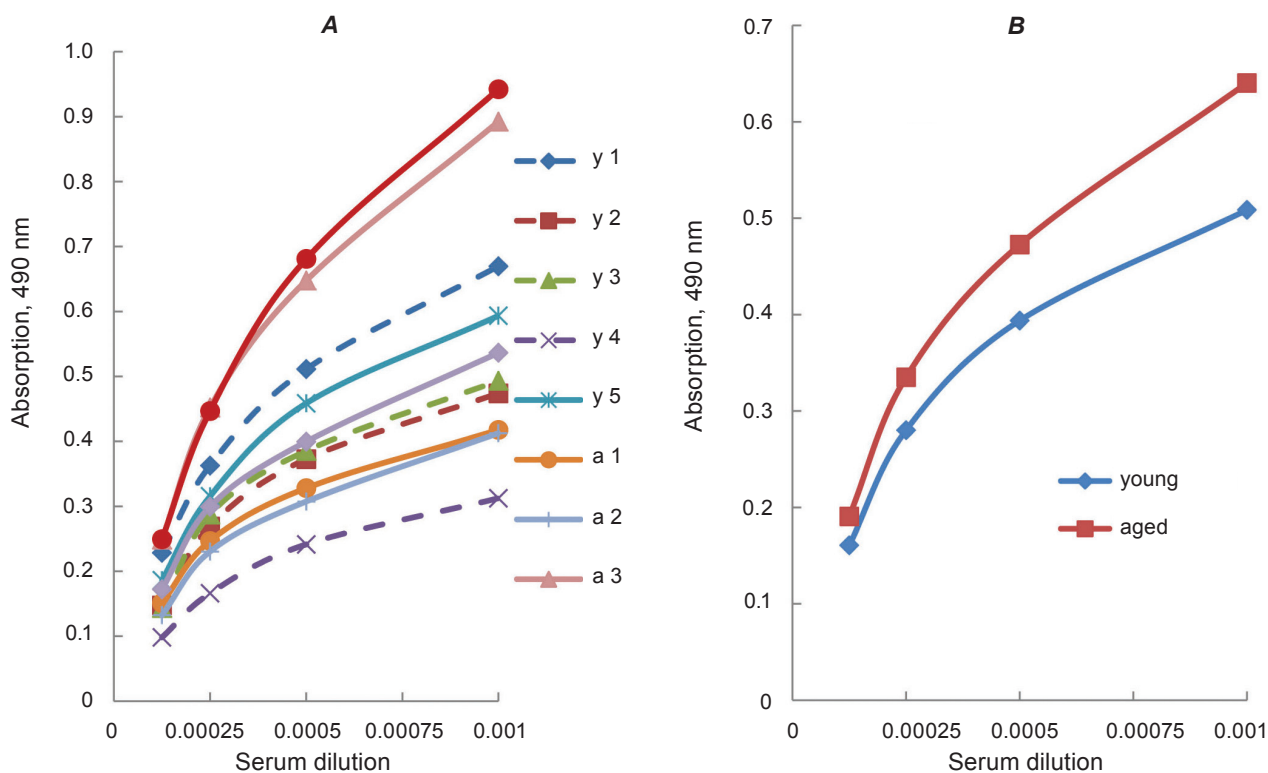


Fig. 2. Titration curves of five serums of young (y) and five serums of aged (a) people (A) for content of IgA PRIG, as well as average values of obtained magnitudes (B)

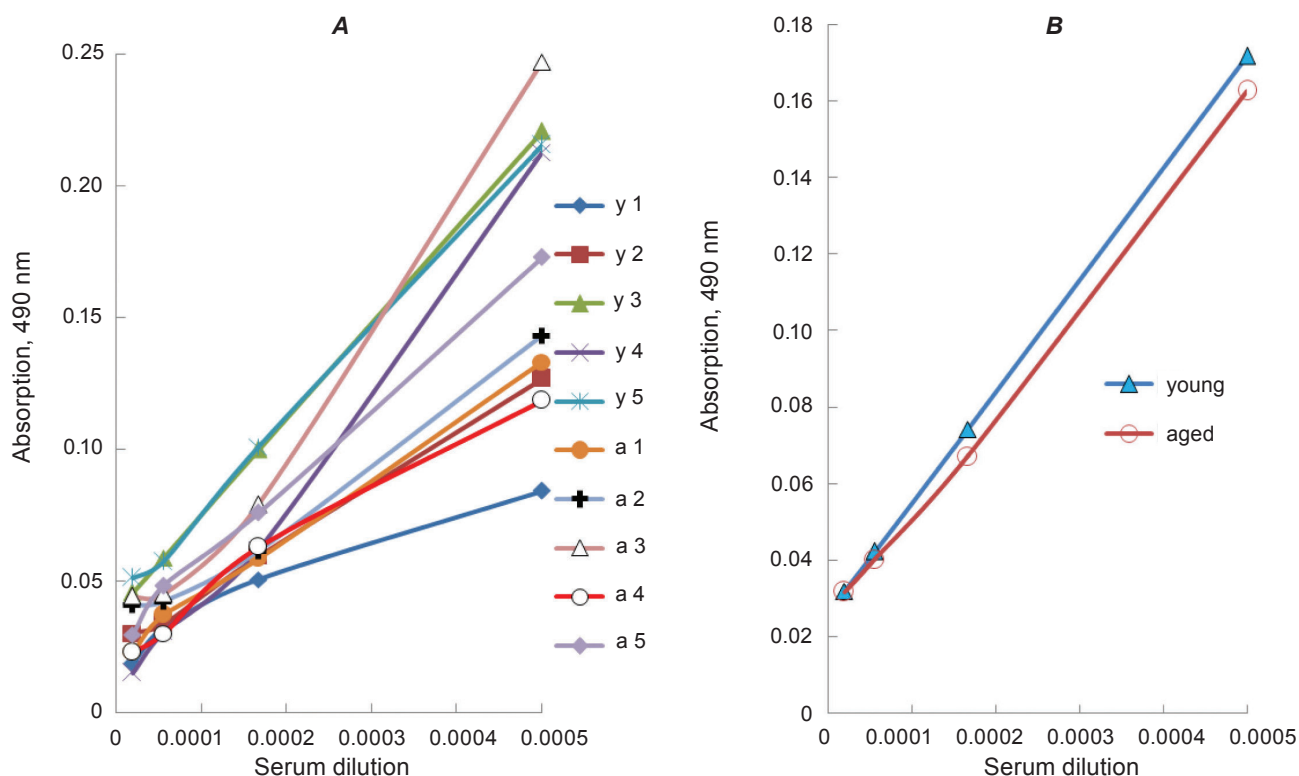


Fig. 3. Titration curves of five serums of young (y) and five serums of aged (a) people (A) for content of IgM PRIG, as well as average values of obtained magnitudes (B).

belonging to different immunoglobulin classes, may prove the importance of functional role of these immunoglobulins, which is yet to be clarified.

### ВІКОВІ ЗМІНИ АКТИВНОСТІ СИРОВАТКОВИХ ПОЛІРЕАКТИВНИХ ІМУНОГЛОБУЛІНІВ (ПРИГ) У ЛЮДЕЙ

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Встановлено, що активність сироваткових поліреактивних імуноглобулінів (ПРИГ) змінюється з віком у практично здорових людей віком від 25 до 70 років. При цьому найбільше (приблизно в 3–4 рази) змінюється активність сироваткових IgG ПРИГ в бік збільшення, тоді як активність IgM ПРИГ, навпаки, не підвищується, а іноді з віком навіть знижується. Активність сироваткових IgA ПРИГ варіює значно більше, ніж активність IgG ПРИГ, і до того ж активність IgA ПРИГ набагато менше залежить від віку, ніж

IgG ПРИГ. Вікові зміни активності сироваткових ПРИГ у людей, що належать до різних класів імуноглобулінів можуть свідчити про важливу функціональну роль цих імуноглобулінів, яку ще слід виявити.

Ключові слова: активність поліреактивних IgG, вікові зміни, ELISA.

### ВОЗРАСТНЫЕ ИЗМЕНЕНИЯ АКТИВНОСТИ СЫВОРОТОЧНЫХ ПОЛІРЕАКТИВНЫХ ИМУНОГЛОБУЛИНОВ (ПРИГ) У ЛЮДЕЙ

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Установлено, что активность сывороточных полиреактивных иммуноглобулинов (ПРИГ) изменяется с возрастом у практически здоровых людей в возрасте от 25 до 70 лет. При этом больше всего (примерно в 3–4 раза) увеличивается

активность сывороточных IgG ПРИГ, тогда как активность IgM ПРИГ, наоборот, не повышается, а иногда с возрастом даже снижается. Активность сывороточных IgA ПРИГ варьирует значительно больше, чем активность IgG ПРИГ и к тому же активность IgA ПРИГ гораздо меньше зависит от возраста, чем IgG ПРИГ. Возрастные изменения активности сывороточных ПРИГ людей, принадлежащих к разным классам иммуноглобулинов, могут свидетельствовать о важной функциональной роли этих иммуноглобулинов, которую еще предстоит определить.

**Ключевые слова:** активность полиреактивных IgG, возрастные изменения, ELISA.

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