Biomedicine & Aging Pathology 3 (2013) 115–118

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EM|consulte
www.em-consulte.com/en

Original article

The reactivity of human serum natural autoantibodies with certain autoantigens increases along with aging

Mir Hadi Jazayeri [a], Ali Akbar Pourfathollah [a,*], Mohammad Javad Rasaee [b], Mohammad Farhadi [c], Narges Zarei [d], Mohammad Ebrahim Jafari [e]

[a] Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Department of Molecular Biology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department and Research Center of Ear, Nose, Throat, Head & Neck Surgery and Related Sciences, Iran University of Medical Science, Iran
[d] Department of Science technology park, Tehran University, Tehran, Iran
[e] Eye Research Center, Rassoul Akram Hospital, Tehran University of Medical Sciences, Iran

ARTICLE INFO

Article history:
Received 17 June 2013
Accepted 28 June 2013
Available online 2 August 2013

Keywords:
Natural autoantibody (NAA)
Autoantigen
Aging
Mean immune reactivity (MIR)

ABSTRACT

Introduction: Natural autoantibodies (NAAs) that recognize autoantigens are mainly generated in the absence of any apparent immunization with foreign antigens (Ags). These antibodies maintain the body homeostasis through functions, such as inhibition of tumor angiogenesis, detection of structural changes in autoantigens, and exertion of anti-inflammatory impacts. The human body is shown to go through immunological and physiological changes during aging. However, data regarding the potential variations in the binding activity of NAAs is still scarce. Therefore, in this study, we were about to explore the trend through which the reactivity of serum NAAs with several autoantigens varies with advancing age.
Materials and methods: Serum samples were prepared from healthy individuals of seven age intervals: (0) cord blood, (1) infancy, (2) childhood, (3) adolescence, (4) early adulthood, (5) middle adulthood, and (6) late adulthood (the elderly). The mean immune reactivity (MIR) of the sera with 24 human autoantigens that were obtained from Immunculus research center (Russia) was determined using ELISA and inter-group comparisons were also performed.
Results: In general, the MIR of serum natural antibodies with the autoantigens was shown to follow an upward trend with advancing age so that the lowest and highest MIRs were detected in the cord blood and late adulthood samples, respectively. Moreover, the results of the inter-group comparisons indicated that the MIRs of the first, second, fourth, and sixth groups were significantly higher than those of their previous groups, i.e. zero, first, third, and fifth groups, respectively.
Conclusion: This study showed that the reactivity of human tissue-specific and non-specific NAAs with autoantigens varied along with aging. Regarding the crucial roles NAAs play in maintaining the body homeostasis, the variation in their concentration at different age intervals might account for the immunological and pathological changes that occur in the elderly.

1. Introduction

Natural antibodies (NAbs) exist in normal human sera in the absence of any apparent immunization with antigens (Ags). A large number of these Abs, called natural autoantibodies (NAAs), have been shown to react with certain autoantigens [1]. These Abs are involved in the clearance of apoptotic cell-derived autoantigens form blood, thereby, reducing the risk of autoimmunity [2]. Natural autoantibodies are reported to play additional roles, including inhibition of tumor angiogenesis [3] and exertion of anti-inflammatory effects [4]. In general, NAAs are suggested to act as housekeepers that not only maintain the whole body homeostasis, but also they serve as disease predictors the level of which in different tissues can inform us about general body health [5,6].

In 1989, Cohen et al. for the first time put forward a hypothesis called “Immunological Homunculus” in support of the presence of NAAs in the body. To prove their theory, Cohen et al. utilized a microarray test (antigen-chip) [7] using hundreds of autoantigens to assess the level of autoantibodies. They termed these autoantigens “biomarkers” with the potential to provide valuable information about the human body state [8]. This was considered as a novel method for identification of potential pathological variations in their primary stages. In 2002, Poletaev coined the term

* Corresponding author. PO Code: 1411713116, Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
E-mail address: pourfa@modares.ac.ir (A.A. Pourfathollah).

2210-5220/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.biomag.2013.06.001

Table 1
The list of the 24 autoantigens.

dsDNA	ANCA	ItM-07	TSH-R
beta2-GPI	KiM-05	HeS-08	AdrM-D.C
Fc	KiS-07	HMMP	Spr-06
CoM-02	LuM-02	Insulin	S100
betaAR	LuS-06	Ins-R	GFAP
TrM-03	GaM-02	TG	MBP

dsDNA: double strand DNA; beta2-GPI: beta 2-glycoprotein I; Fc: fragment of IgG; CoM-02: myocardial Ag; betaAR: beta 1 adrenoreceptors; TrM-03: thrombocyte (platelet) Ag; ANCA: vascular endothelium Ag; KiM-05: kidney membrane Ag; KiS-07: kidney cytoplasmic Ag; LuM-02: lung membrane Ag; LuS-06: lung cytoplasmic Ag; GaM-02: stomach membrane Ag; ItM-0713: small intestine membrane Ag; HeS-08: liver cytoplasmic Ag; HMMP: liver mitochondria Ag; Insulin: Insulin-secreting; TG18: insulin receptors; TG: thyroglobulin; TSH-R: TSH-receptor; AdrM-D.C: adrenal gland Ag; Spr-06: spermatozoid Ag; S100: S100 protein; GFAP: glial filament astrocyte protein; MBP: myelin basic protein.

"Immunculus", derived from Immunological Homunculus. In his viewpoint, NAAs served as a magic mirror reflecting the physiological, immunological, and pathological state of each individual's body state. In fact, the pattern of these Abs, similar to that of the fingerprints, is proposed to be specific to each person [9]. Moreover, Notkins proposed natural autoantibodies as biomarkers that are also capable of maintaining the body homeostasis, and preventing immunological distortions [10,11].

Based on the existing theories, the level and pattern of NAAs undergo certain age-related fluctuations, leading to deviation of total body system from a physiologic to a pathologic state with advancing age. It has been shown that the amount of these Abs increases with age, proposing them as crucial factors in the old age [12]. The pattern of NAAs in each individual's body is influenced by several factors: age, gender, race, nutrition, and environment [13–16]. Here, we used a panel of 24 autoantigens to determine and compare the pattern of NAAs in the sera of 270 normal individuals categorized in seven separate age groups.

2. Materials and methods

2.1. Blood samples and groups

A total of 270 normal blood samples were obtained under a physician's supervision and categorized into seven distinct age groups: cord blood, infancy (under 1 year old), childhood (1 to 12 years old), adolescence (13 to 19 years old), early adulthood (20 to 39 years old), middle adulthood (40 to 64 years old), and late adulthood (older than 65 years old) [17]. Volunteers were confirmed to have no evidence of major clinical problems and donors with issues,such as infection, allergies, high blood pressure, and diabetes mellitus were not included in the study. Blood samples were collected between March, 2011 and April, 2012 at Military Retirees Association, Shahid Beheshti University, Tarbiat Modares University, Boys and girls schools of Tarbiat Modares University, children medical center of Imam Khomeini Hospital, and cord blood Section of Tehran Blood Transfusion Center. The blood donors were informed about the study procedure and aim, and written consent was obtained by the medical staff. All the protocols were approved by the ethical committee of medical faculty of Tarbiat Modares University.

2.2. Autoantigens

Twenty-four autoantigens (Table 1) were purchased from The Medical Research Center "Immunculus" (Moscow, Russia) and used in the ELISA experiments [18].

2.3. Measurement of the level of serum natural autoantibodies

Blood specimens were collected into sterile tubes (Improve, Belgium), and their obtained sera were assessed for the level of natural autoantibodies against the 24 human autoantigens using ELISA. Each sample serum was diluted by 1/200 and the control serum (obtained from Russia) depending on the type of autoantigen was diluted by 1/25 to 1/600. The optical density (OD) of wells was measured and analyzed using an ELISA Reader (STAT FAX 2100 Microplate Reader, USA).

2.4. Mean immune reactivity calculation method

Mean immune reactivity (MIR) of sera was determined based on the formula devised by Schoenfeld [19]:

$$IR = (R–X(Fab) \times 100)/R – St(Fab) – 100(R – X(Fab) = OD)$$

After determining the OD of either standard (Cont) or donor samples (Ag), immune reactivity (IR) and MIR were calculated according to the below formulas. For a single Ag (e.g. Ag1) the formula was follows:

$$IR_{Ag1} = (ODAg1 \times 100)/ODCont1 – 100$$

And for n Ags:

$$IR_{AgN} = (ODAgN \times 100)/ODCont\ N – 100$$

MIR of each individual's serum with 24 autoantigens was calculated as follows:

$$MIR = \sum_{n=1}^{24} \frac{IR(AgN)}{24}$$

The obtained values were normalized through calculating the relative activity of each Ag versus that of the total Ags according to the following formula:

$$R_{AG1}^{norm} = R_{AG1} - MIR$$

through

$$R_{AGn}^{norm} = R_{AGn} - MIR$$

Where $n = (1, 2, \ldots, 24)$ in the current study.

2.5. Statistical analyses

GraphPad Prism (version: 5.04) was used to perform statistical analyses. The comparison of separate age groups with regard to their MIR was carried out using one-way ANOVA. P values less than 0.05 were interpreted as statistically significant. The data were represented as mean ± SEM.

3. Results

Human lifetime is either divided into decades or into six groups. The latter division pattern is defined by Word Health Organization (WHO), and consists of:

- infancy (under 1 year old);
- childhood (1 to 12 years old);
- adolescence (13 to 19 years old);
- early adulthood (20 to 39 years old);
- middle adulthood (40 to 64 years old);
- late adulthood (older than 65 years old) groups.

Due to the significant differences observed between cord blood samples and those obtained from under 1-year infants, in the