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Research paper

Immune reactivity in rats selected for the enhancement or elimination of aggressiveness towards humans

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HIGHLIGHTS

- Genetic differences in aggression are related to differences in immune reactivity.
- Blood $CD4^+/CD8^+$T lymphocyte ratio is higher in aggressive rats than in tame rats.
- Aggression is associated with increased level of MCP-1 before and after immunization.
- Antigen increased IL-1α level in aggressive rats but decreased it in the tame rats.
- IgM-immune response to SRBC is higher in aggressive rats than in tame rats.

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ABSTRACT

This study analyzes immune reactivity in two lines of rats selected for the enhancement or elimination of aggressiveness toward humans. Compared to nonaggressive line, aggressive rats showed increased blood ratio of $CD4^+$ and $CD8^+$T lymphocytes, monocyte chemoattractant protein (MCP)-1 level both before and after immunization with sheep red blood cells (SRBC), enhanced IgM-immune response, as well as decreased level of interleukin (IL)-1α before immunization. However, antigen administration produced IL-1α increase in aggressive rats and its decrease in nonaggressive rats compared to non-immunized rats of the same lines. In addition, line-dependent alterations of T lymphocyte distribution in response to immune activation have been found only in the spleen. It is suggested that genetic differences in aggressive behavior may contribute to differences in immune function.

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1. Introduction

A growing number of clinical and animal studies are indicating that different forms of aggressive behavior are related to altered immunological responsiveness [1–11]. Individuals high in aggression, hostility and anger have been found to be at increased risk for immune dysfunction and inflammatory diseases [9,11]. It has also been shown that human aggression is positively associated with enumerative measures of cellular immunity including the number of blood helper/inducer $CD4^+$T cells [6]. Aggressive mice exposed to chronic social stress demonstrated an increased immune response accompanied with accumulation of $CD4^+$T lymphocytes in the bone marrow [1,3–5,7]. There is also evidence that genetic factors contribute both to specific behavioral responses and immune reactions [8]. However, there is little evidence for a linkage between a genetic predisposition toward developing aggressive behavior and immune processes, especially when they are coupled with an immune challenge. Only few studies are indicating that differences in social behavior in mice selected for high or low social isolation-induced aggression may affect state-related changes in some measures of innate immunity [8].

Strong individual differences in aggressive behavior have been obtained at the Institute of Cytology and Genetics (Novosibirsk, Russia) by selective breeding of Norway rats (Rattus Norvegicus) for

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either high levels or for the lack of fear-induced aggression. To date these rat lines have been extensively characterized in terms of their behavior, physiology and neurochemistry. The aggressive rats differ from nonaggressive line not only in defensive aggression but also in other behavioral parameters, which include intermale aggression, higher levels of anxiety and emotionality, stressed reactivity, the decreased exploratory activity [12–15]. In addition, line differences have been found in activities of the hypothalamic-pituitary-adrenal (HPA) axis and neuromediator systems [12–18], which are involved in the mechanisms controlling aggression [19–20], as well as in neuroimmunomodulation [21–26].

In order to analyze the association between genetically determined differences in aggressive behavior and immune reactivity, we examined the content of different T lymphocyte subpopulations in the blood and spleen, levels of peripheral cytokines before and after immunization with sheep red blood cells (SRBC), the numbers of IgM-antibody forming cells (IgM-AFC) in the spleen in two rat lines selected for the enhancement or elimination of aggressiveness towards humans.

2. Materials and methods

2.1. Animals

The experiments were performed in adult male rats (2–3 months) which were selected for either enhanced or reduced (tameness) aggression towards humans over 70 generations. The rats were bred and maintained under standard vivarium conditions at the Institute of Cytology and Genetics (Novosibirsk, Russia). They were housed in standard ($50 \times 40 \times 30$ cm) cages under a natural light–dark cycle, corresponding to the outside conditions. Food and water were available *ad libitum*. All procedures were performed in accordance with principles of the declaration of Helsinki, and after Local Ethical approval by Scientific Research Institute of Physiology and Basic Medicine, Institute of Cytology and Genetics SB RAS.

2.2. Glove test

The level of aggression/tameness was measured in the "glove test" by confronting rats of both lines with an approaching human hand and attempting to handle them [12,13,17]. The intensity of response to handling was evaluated according to the following five-score system: 0—rat permits to handle and does not make any attempts of avoiding; 1—permits to handle and makes evasive movements in the hand; 2—moves away from the hand and while being picked up tries to break loose; 3—actively escapes handling and while being picked up, rat can emit loud screaming noises, opens mouth or bites; 4—rat does not permit to handle, attacks the hand and emits loud screaming noises. In our experiments we have used rats, which were characterized by either extremely high aggression (4 points according to the scale) or by a complete lack of aggressiveness (0 points according to the scale). The analysis of immune parameters was performed within 2–3 weeks after behavioral testing.

2.3. Immunization

Rats of both lines were immunized with a T-cell-dependent antigen-sheep red blood cells (SRBC), which were suspended in saline and were injected intraperitoneally at 5×10^8 per 0.5 ml. This dose of SRBC is known to be sufficient to induce humoral immune response [1,4,5,10,28] mediated by $CD4^+$T helpers of type 2, which are involved in B cell activation [27].

2.4. Determination of T lymphocyte subpopulation

2.4.1. Monoclonal antibodies

T lymphocytes subpopulations ($CD3^+$, $CD3^+4^+$ and $CD3^+8^+$) from the peripheral blood and spleen of non-immunized and immunized rats were analyzed by flow cytometry. Cells were stained with the following monoclonal antibodies (MoAb) against surface markers: CD3 (clone G4.18) fluorescein isothiocynate (FITC); CD4 (clone OX-35) phycoerythrin (PE); CD8 (clone OX-8) peridinin-chlorophyll proteins (perCP). All monoclonal antibodies were obtained from BD PharmingenTM (USA).

2.4.2. Cell preparation

Trunk blood was immediately collected after the animals were decapitated into Becton Dickinson tubes containing K3EDTA, the samples were mixed with RPMI1640 medium to adjust cell concentration to 1×10^6/100 μl. The spleen was harvested and cut into several pieces and then disaggregated mechanically into single-cells suspension. The tissue was washed in RPMI-1640 medium and passed through a 70 μm cell strainer. The suspension was washed twice again in RPMI1640 medium at 200 g for 5 min. The cell pellet was resuspended in RPMI1640 medium, adjusted to a concentration of 1×10^6/100 μl and placed in the 96-well flat-bottomed plate in a volume of 100 μl in each well.100 μl of the blood or spleen suspension was added to appropriate amounts of fluorescently conjugated primary or secondary antibody (isotypic control) and incubated for 20 min in the dark at room temperature. Erythrocytes of the blood and spleen were lysed with 1.0 or 0.1 ml BD FASCTM Lysing Solution (Becton Dickinson, USA), respectively, for 15 min at room temperature in darkness. Thereafter, the cells were washed three times with phosphate buffered saline (PBS) at 200 g for 5 min. The cells were then fixed by adding 0.5 ml of 1% paraformaldehyde to each tube.

2.4.3. Flow cytometry

Samples were analyzed on a FACS CANTOTM II flow cytometer (Becton Dickinson, USA) equipped with 3 lasers, blue 488 nm, a red 633 nm, and a violet 405 nm. Data processing was carried out using the FACS Diva 6.1.2 software. A minimum of 3000 lymphocytes in each probe was analyzed.

2.5. Cytokine analysis

Serum from non-immunized and immunized rats used for cytokine analysis was prepared by collecting blood into tubes and allowing it to clot for 10 min at room temperature. Then, it was centrifuged (1500 rpm) for 15 min at room temperature. Serum was collected and stored at −20 °C. Cytokines were detected in unstimulated with mitogens samples using Rat Cytokines 5plex Kit FlowCytomix (BMS826FF) on a FACS CANTOTM II flow cytometer (Becton Dickinson, USA). For surface markers, the following specific anti-rat MoAb were used: IFNγ, IL-1α, IL-4, tumor necrosis factor (TNF)-α, and monocyte chemoattractant protein (MCP)-1). The assays were run according to the standard manufacturer's protocol. A mixture of fluorescent beads coated with specific MoAb for each analyte was added in tubes containing either 25 μl of sample or preliminary diluted standard mixture. After that 50 μl of biotin-conjugated second antibody mixture was added. Tubes were then incubated for 2 h in the dark at room temperature. After incubation, samples were washed twice in 1 ml of Assay buffer (PBS with 10% bovine serum albumin), tubes were centrifuged at $200 \times g$ for 5 min, and supernatants were removed carefully. In all tubes 50 μl of streptavidin-phycoerythin (PE) solution was added followed by incubation for 1 h in the dark at room temperature. The samples were washed twice in 1 ml of Assay buffer and centrifuged at $200 \times g$ for 5 min. After addition of Assay buffer (200 μl)

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