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Postmortem changes in hapten-specific IgE antibody responses in mice

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Summary. This Study was carried out to examine the post-mortem stability of hapten-specific immunoglobulin E (IgE) antibody in mice by the passive cutaneous anaphylaxis test in rats. In vitro IgE antibody responses in blood were stable for 3 days at room temperature. Even in cases when blood could not be obtained from the cadaver it was possible to measure the hapten-specific IgE antibody response up to 3 days after death using the supernatant of the visceral homogenates.

Key words: Post-mortem change – Immunoglobulin E – Passive cutaneous anaphylaxis – Viscera – Mouse

Zusammenfassung. Die Untersuchung wurde durchgeführt, um mit Hilfe des passiven kutanen Anaphylaxie-Tests bei Ratten die postmortale Stabilität des haptenspezifischen Immunoglobulins E von Mäusen zu überprüfen. In-vitro IgE-Antikörper-Antworten waren – unter Raumtemperatur-Bedingungen – 3 Tage unverändert. Selbst in Fällen, wo Blut vom Kadaver nicht gewonnen werden konnte, war es möglich, die hapten-spezifische IgE-Antikörper-Antwort bis zu 3 Tagen nach dem Tode zu bestimmen, indem der Überstand von Organ-Homogenaten verwendet wurde.

Schlüsselwörter: Postmortale Veränderungen – Immunoglobulin E – Passive kutane Anaphylaxie – Organe – Maus

Introduction

Fresh or stored frozen serum is generally used for measurement of antibody responses. However, in the field of forensic medicine it is not always possible to obtain serum, or even whole blood, because of post-mortem degeneration, as autopsies are sometimes performed some days after death. Therefore, the antibody response is not often measured in post-mortem cases. Measurement of the hapten-specific antibody response in post-mortem tissue would be useful for determining causes of death related to the immune system (e.g. anaphylaxis from immune reaction) with hapten-specific IgE antibody and antigen.

In this paper, we describe the stability of hapten-specific IgE antibody, which was produced in mice by immunization with the hapten 2,4-dinitrophenyl (DNP) conjugated to keyhole limpet haemocyanin (KLH), both in vitro and in cadaver viscera stored at room temperature for 3 days.

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Materials and methods

1. Hapten-protein conjugates. KLH, bovine-γ-globulin (BGG) and ovalbumin (OVA) were obtained from Aldrich (Milwaukee, USA), Sigma (St. Louis, USA), and Funakoshi (Tokyo, Japan), respectively. 2,4-Dinitrobenzenesulfonic acid sodium salt was obtained from Eastman Kodak (New York, USA). The DNP conjugates, $DNP_{12.4}$-KLH, $DNP_{12.3}$-BGG, and $DNP_{5.3}$-OVA were prepared by the method described by Katz et al. [1]. Subscripts refer to the number of moles of DNP per 100,000 molecular weight units of KLH, per mole of carrier for BGG and OVA.

2. Animals. Female A/J mice and male Wistar rats were obtained from Japan SLC and KYUDO, respectively. The mice were 7 weeks old when first immunized and the rats were 8 weeks old when used for the PCA test.

3. Immunization. Forty mice received primary immunization with an i.p. injection of $DNP_{12.4}$-KLH (15 μg) mixed with 5 mg of $Al(OH)_3$ gel and saline in a total volume of 0.5 ml. $Al(OH)_3$ gel (alum) was prepared by mixing equal volumes of 2 N $Al_2(SO_4)_3$ and 2 N NaOH as described by Levine and Vaz [2]. Mixtures of hapten-protein conjugates with alum were prepared immediately before use. After 4 weeks, all mice received secondary immunization with the same dose of antigen-alum mixture.

4. Sampling. All mice were killed by inhalation of ether 7 days after the second immunization and divided into four groups (I–IV) of 10.

For the first group (I), blood was drawn by cardiac puncture soon after death. Some of the blood was transferred to tubes containing 0.1 ml 1% EDTA.2K (anti-coagulant), and mixed gently (whole blood sample). The remaining blood was centrifuged (1800 *g*

for 10 min) and the supernatant removed (plasma sample). Both samples (whole blood and plasma) were divided among four tubes: the first (I-1) was strored immediately at −20°C; the second (I-2) was left for 1 day at room temperature (approximately 20°C) then stored at −20°C; the third (I-3) was left for 2 days; and the fourth (I-4) was left for 3 days, then stored at −20°C until tested. (I-1 to I-4 were in vitro samples.) The heart, lungs, liver, kidneys, and spleen were removed, accurately weighed and placed in tubes containing 0.4 ml 1% EDTA.2K solution. Samples were homogenized, centrifuged (8000 *g* for 30 min) at 4°C and the supernatant removed and stored at −20°C.

The second group of mice (II) was left at room temperature for 1 day after death; the third (III) was left for 2 days; and the fourth (IV) for 3 days. Each organ was removed and the sample preparation was performed as for the first group (I). Blood could not be obtained from the mice in groups II, III and IV because of postmortem clotting.

5. Measurement of anti-DNP IgE antibodies. The titres of IgE anti-DNP antibodies was determined by the passive cutaneous anaphylaxis (PCA) reaction using rats as described by Levine and Vaz [2]. Blood samples and supernatants from viscera were serially diluted (2-fold) in saline. From each sample dilution, 100 µl was injected i.d. into the dorsal skin of the test rat. Twenty-four hours after i.d. sensitization, DNP-specific PCA reactions were elicited by i.v. injection of 1 mg $DNP_{12.3}$-BGG in 1 ml Evans blue dye (1%) dissolved in saline. The reactions were noted and recorded as the reciprocal of the highest dilution of blood evoking threshold PCA reactivity (5 mm diameter), 30 min after the i.v. injection.

Results

Hapten-DNP-specific IgE antibody was clearly detected by the PCA test in rat plasma taken 1 week after the second antigen challenge. In preliminary investigations, A/J mice were immunized with the same dose of $DNP_{5.3}$-OVA, or $DNP_{12.3}$-BGG, and using the same schedule as $DNP_{12.4}$-KLH. Hapten-DNP-specific IgE antibody was detected in approximately the same titre as KLH carrier protein. The patterns of changes in IgE antibody titres did not much differ among the different carrier proteins used for immunization or between male and female mice

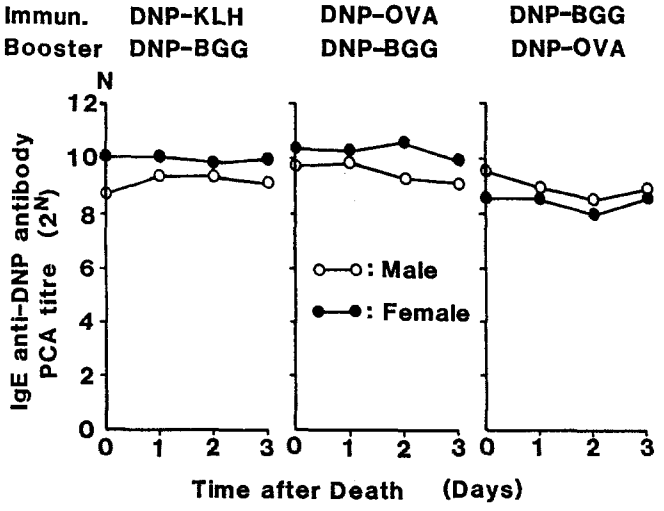

Fig. 1. Postmortem changes in PCA titres of hapten DNP-specific IgE antibody in plasma in vitro. Each plot was obtained from the means of 10 actual titres

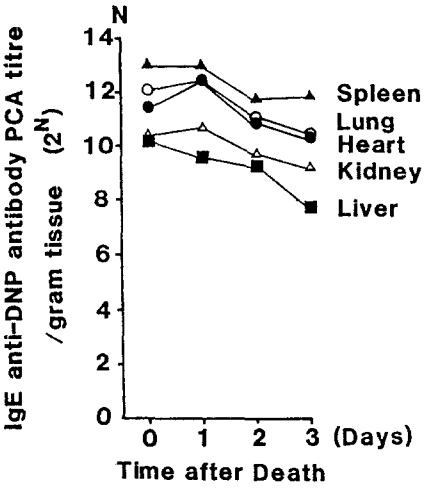

Fig. 2. Postmortem changes in PCA titres of DNP-specific IgE antibody in cadaver viscera. Each plot was obtained from the means of 10 actual titres

(Fig. 1). Detailed investigations were then performed with $DNP_{12.4}$-KLH in female A/J mice.

No difference in the pattern of response of DNP-specific IgE antibody was found between plasma and whole blood in vitro when left at room temperature (approximately 20°C) for 3 days (data not shown). It was impossible to draw blood by cardiac puncture on the day after death, and blood data were therefore limited to the in vitro study results.

The mice gradually decomposed and by 3 days after death, the stomach and intestines were inflated with gas from putrefaction, and post-mortem decomposition was present in every organ. However, DNP-specific IgE antibodies could be detected by the PCA test in the supernatant of the tissue homogenates. Figure 2 shows the postmortem titres in each organ 3 days after death. The titres are expressed per gram of wet tissue. The titres 3 days after death were slightly lower. DNP-specific IgE antibody responses could be accurately measured using the supernatant from each organ up to 3 days after death.

Discussion

A/J mice were used for this experiment because this strain has a high rate of IgE antibody production [3]. The immunization and sampling schedule was based on the results of Hamaoka et al. [4] which showed that the anemnestic response peak of hapten-specific IgE antibody appeared 7–10 days after the second antigen challenge. The PCA test was performed for the measurement of DNP-specific IgE antibody because this method using rats can specifically analyse mouse IgE, without cross-reaction to mouse IgG antibody and has a high sensitivity [5].

Fresh or stored frozen antiserum is generally used for biochemical research. There have been no reports about changes of antibody titres after being left at a certain temperature and there has been no research on postmortem changes in antibody titre. In this experiment

using mice, it appears that DNP-specific IgE antibody in serum and whole blood in vitro left at room temperature can be analysed up to 3 days after death with no change in titre and may be detected in internal organs in cases in which blood cannot be obtained from the cadaver. Therefore IgE antibody is almost completely stable for at least 3 days at room temperature in spite of post-mortem cytochemical degeneration. This suggests that hapten-specific IgE antibody may be detected from human cadaver tissue in approximately the same titre as immediately after death. In post-mortem samples it may therefore be possible to identify hapten-specific IgE, which shows correlation with acute hypersensitivity reactions (type I) and provides evidence of death from anaphylaxis.

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References

1. Katz DH, Paul WE, Goidl EA, Benacerraf B (1970) Carrier function in anti-hapten immune responses: I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. J Exp Med 132:261–282
2. Levine BB, Vaz NM (1970) Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. Int Arch Allergy Appl Immunol 39:156–171
3. Chiorazzi N, Fox DA, Katz DH (1976) Hapten-specific IgE antibody responses in mice: VI. Selective enhancement of IgE antibody production by low doses of X-irradiation and by cyclophosphamide. J Immunol 117:1629–1637
4. Hamaoka T, Katz DH, Bloch KJ, Benacerraf B (1973) Hapten-specific IgE antibody responses in mice: I. Secondary IgE responses in irradiated recipients of syngenetic primed spleen cells. J Exp Med 138:306–311
5. Mota I, Wong D (1969) Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. Life Sci 8:813–820