Shimane J. Med. Sci., Vol.9, pp.29-35, 1985

STUDIES ON HETEROPHILE ANTIBODY BY RABBIT HEMAGGLUTINATION TEST IN VARIOUS DISEASES OF THE ORAL MUCOSA

(heterophile antibody/rabbit hemagglutination test/oral mucous disease)

Toshio HARADA, Hirofumi KISHIMOTO, Takahide SUGIHARA and Yasuro YOSHIMURA

Department of Oral and Maxillofacial Surgery, Shimane Medical University, Izumo 693, Japan

(Received June 13, 1985/Accepted July 24, 1985)

Preliminary studies were performed on heterophile antibodies by rabbit and sheep hemagglutination tests in various oral mucous diseases. On rabbit hemagglutination test, most sera from normal healthy persons showed titers from 256 to 512, while the test on sheep revealed titers from 64 to 128. These titers roughly paralleled each other, although the latter showed a titer of 2^2 lower than the level of the former. On the other hand, the titer of patients with lichen planus or oral candidiasis on these tests showed a level close to normal. Patients with aphthous stomatitis or recurrent aphthous ulcer revealed a fairly lower than normal titer on both tests. From the results of an absorption test using sediment of kidney or erythrocyte corpuscle of various species, it was suggested that the heterophile antibody on the rabbit hemagglutination test did not belong to neither the Forssman, Paul-Bunnell(P-B) or serum sickness (Hanganutziu- Deicher; H-D) types.

The etiology of most lesions in the oral mucous membrane still remains obscure; however they have been becoming gradually clear from an immunological point of view. Recently it has been shown that herpetic diseases or candidiasis may develop through immunodeficiency of a T cell, and that immunodeficiency of a B cell may produce stomatitis or ulcer formation in the oral mucosa

(1). In this preliminary study, we also explored the possibility of an immunologic factor in the diseases of the oral cavity by estimating the level of serum heterophile antibodies.

MATERIALS AND METHODS

The sera of healthy persons[21]([ ]; Number of cases) and of patients with various diseases, such as aphthous stomatitis[20], recurrent aphthous ulcer[16], oral lichen planus[13], oral candidiasis[7], Sjögren's syndrome[8], jaw bone lesions[16], oral benign tumor[13] and oral cancer[16] were stocked in a deep frozen condition at -80°C until needed for experiments. Diagnosis was confirmed not only by clinical findings, but also by biopsies. For the purposes of data tabulation, patients with aphtha formation were classified into two different groups; aphthous stomatitis and recurrent aphthous ulcer according to International Classification of Diseases (2).

Rabbit (or sheep) hemagglutination

The experimental procedure was carried out according to the modified Davidsohn's method (3), namely, serum samples were heated at 56°C for 30 min before examination, and serial dilutions of serum in saline (volume 0.5ml) were mixed with 0.5ml of 1% suspension of rabbit (or sheep) erythrocytes which was washed three times with saline. These tubes were incubated at 37°C for 1 hour, they were then left overnight in a cold room, the agglutinated condition was then macroscopically examined after shaking the tubes gently, and the titer was determined by the highest dilution ratio.

Absorption experiments

Homogenized kidney of guinea pig or mouse and erythrocyte of mouse, sheep or rabbit were washed three times with saline. The sediment or erythrocyte corpuscle was mixed with an equal volume of the undiluted serum. The mixtures were incubated by shaking at room temperature for 60 min, and centrifuged at 3,000 r.p.m. for 60 sec. Then the supernatant was recovered. The procedure was repeated from 3 to 5 times. Absorbed serum obtained was measured by rabbit hemagglutination test, and compared with the preabsorbed titer in various sera.

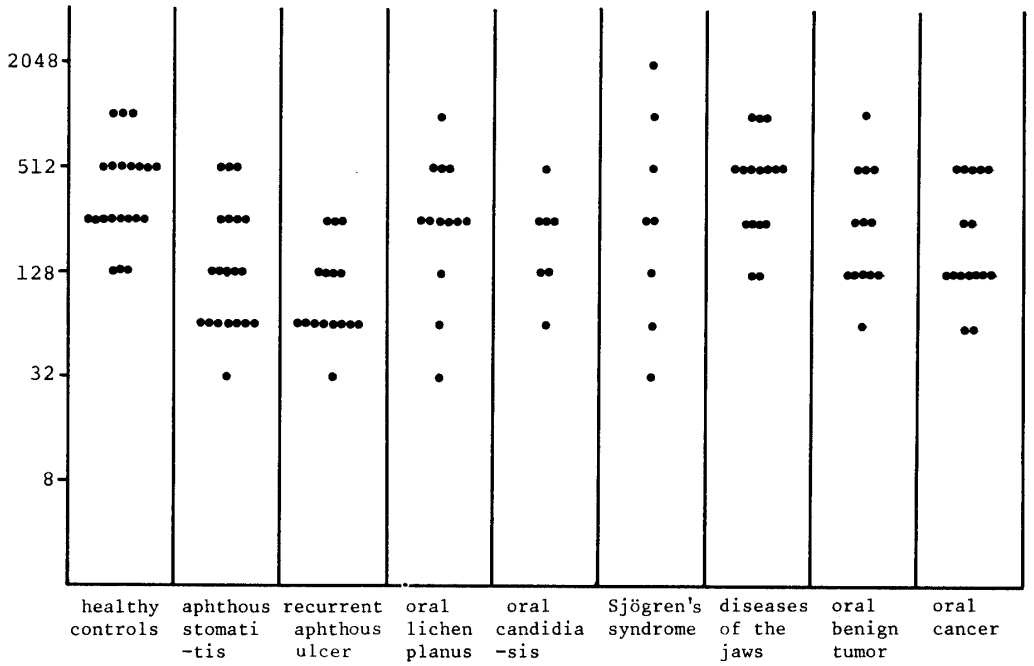

Fig.1. Serum titers on rabbit hemagglutination test in various oral diseases

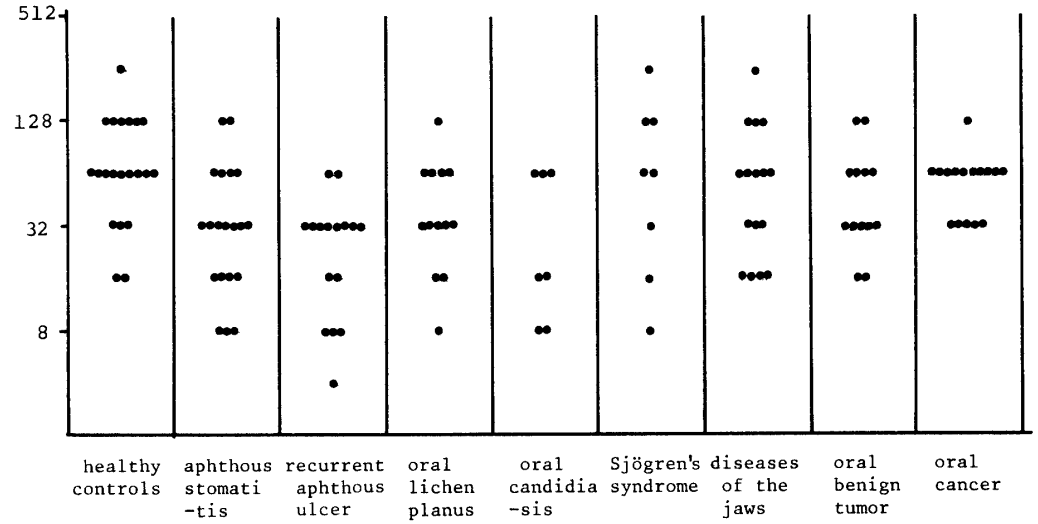

Fig.2. Serum titers on sheep hemagglutination test in various oral diseases

RESULTS

Hemagglutination titers in healthy control sera

Ten males and eleven females were selected as healthy persons with an age range of 22 to 64 years; they had no anamneses of oral mucous diseases or of serum sickness, and had never undergone heteroserotherapy. With regard to the rabbit hemagglutination test, fifteen of these 21 cases (71.4%) showed a titer of 256 to 512, while that of the sheep revealed a titer of 64 to 128 in 15 of these controls (71.4%) (Figs.1 and 2). These

titers roughly paralleled each other, although the latter revealed a titer of about 2^2 less than the former. There were no significant differences in titers according to ageing in the control sera.

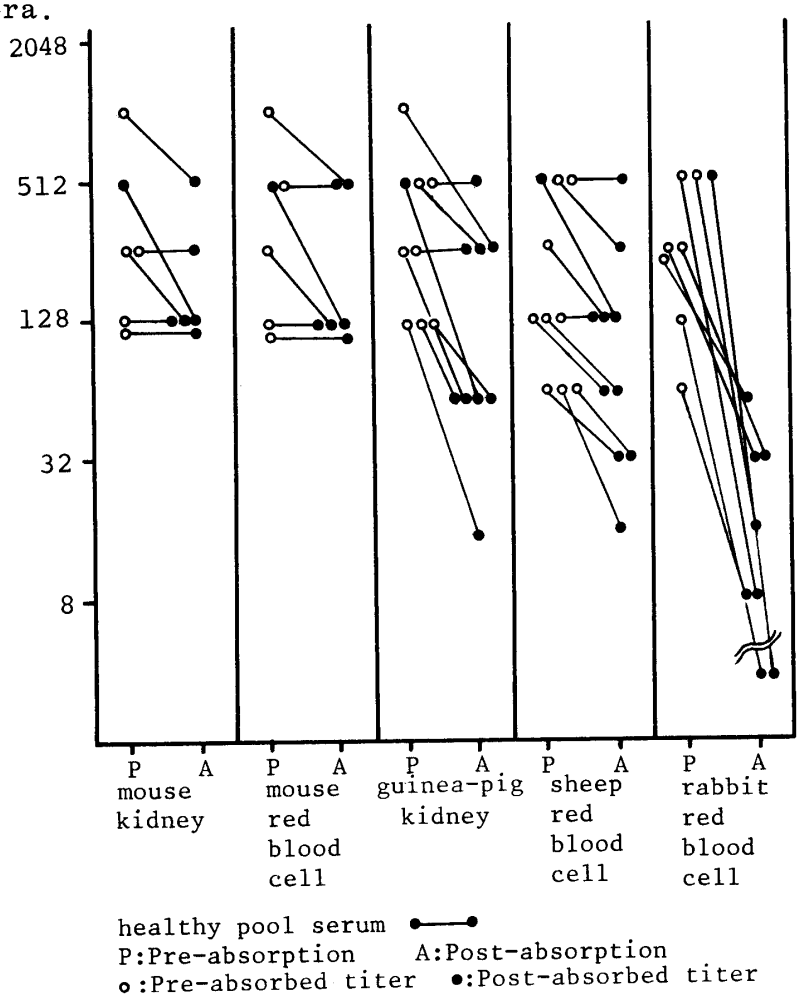

Fig.3. Rabbit hemagglutination titers on various oral diseases using absorption experiments

Hemagglutination titer in various oral diseases

Titers on rabbit and sheep in hemagglutination tests roughly paralleled each other in various patient groups (Figs.1 and 2). The titers of patients with oral lichen planus, oral candidiasis, diseases of the jaw bones and oral benign tumors showed a level close to normal, whereas the patients with aphthous stomatitis or recurrent aphthous ulcer revealed a titer considerably lower than normal on both kinds of hemagglutination test. The titer of oral cancer patients also indicated a level slightly lower than normal. There existed wide variance in the hemagglutination titer of Sjögren's syndrome. Age range of the patients with various oral diseases was from 18 to 78 years, and there were no

Table I. RABBIT HEMAGGLUTINATION TITERS ON HEALTHY POOL SERUM BY ABSORPTION TESTS

	Titers of pre-absorption	Titers of post-absorption
mouse kidney	512	128
mouse erythrocyte	512	128
sheep erythrocyte	512	128
guinea pig kidney	512	64
rabbit erythrocyte	512	8>

Table II. DISTRIBUTION OF HETEROPHILE ANTIGEN

	Sheep erythrocyte	Rabbit erythrocyte	Guinea pig kidney	Mouse erythrocyte
Forssman	+	-	+	+
P-B	+	-	-	*
serum-sickness (H-D)	+	+	+	+

partially modified reference(8)
*unknown so far as author was able to investigate

significant differences in titers according to age.

Rabbit hemagglutination titers using absorption experiments

Absorption experiments using sediment of kidney or erythrocyte corpuscle of various species were carried out in patient and in control sera to estimate the nature of the heterophile antibody on rabbit hemagglutination test. With regard to evaluation it was estimated to be a positive finding when the titer was more than 2^3. As shown in Fig.3, on the test with rabbit erythrocyte, the majority of sera indicated an absorption titer more than 2^3. Whereas, the test with mouse erythrocyte or kidney revealed no absorption in any samples except those of 2^2 on the healthy pool serum, and on the test with sheep erythrocyte, they showed no absorption more than a titer of 2^2. On the test using guinea pig kidney or rabbit erythrocyte, absorption titers of 2^3 or 2^7 were observed in the healthy pool serum (Table I).

DISCUSSION

It is a well-known fact that there are heterophile antibodies to various antigens in human sera, and nowadays they are classified into three groups; the Forssman (4), the Paul-Bunnell(P-B)(5), and the serum sickness (Hanganutziu-

Deicher; H-D)(6) types. The Forssman antigen is widely distributed in animals and plants. However, its clinical significance has not yet been confirmed. P-B antibody shows a high level in infectious mononucleosis and therefore is of great value in the diagnosis of this disease. Serum sickness (H-D) types were found in sera of serum sickness but as their antibody exists in patients who received no injection of a foreign species serum, it is considered that this antibody might result from autoimmunity to H-D antigen (6).

Recently, a rabbit hemagglutination test was used to measure the titer of heterophile antibodies in preference to sheep hemagglutination or lysis of bovine erythrocytes because neither of these latter tests indicated titer levels high enough to enable us to compare various kinds of diseases. So we tried to estimate the nature of heterophile antibody by testing its characteristics on absorption experiments. The test using mouse kidney or erythrocyte revealed no clear discernible absorption in any samples, except those of 2^2 on the healthy pool serum, which suggested that this antibody might differ from the Forssman antibody (as shown in Fig.3, Table II). However, on the test with guinea pig kidney or rabbit erythrocyte, values of 2^3 or 2^7 were observed in the healthy pool serum. And on the test with sheep erythrocyte, they showed no absorption more than a titer of 2^2. Thus it was considered that this antibody may not belong to Forssman, P-B, or serum sickness (H-D) types.

On the other hand, it had been said in previous reports (7, 8) that there were wide differences in hemagglutination titers among various diseases. Kumakawa *et al.* (7) reported that most patients with myeloma indicated low levels on rabbit hemagglutination test, and suggested that the measurement of rabbit hemagglutination titer in various sera might be useful as one of the parameters of humoral immunity. In this study, we observed that more than half the patients with aphthous stomatitis or recurrent aphthous ulcer showed lower titer levels than the normal. From the immunological aspect, we had already reported that the number of B-cells in patients with aphthous stomatitis was fewer ($821 \pm 64/ mm^3$) than in the healthy controls ($1034 \pm 208/ mm^3$) (9). A relationship between the lower titer of heterophile antibodies on rabbit hemagglutination test and suppressive humoral immunity may be suggested from our separate experiments described above.

This work was supported by a Grant-in-Aid (No.58771446) from the Ministry of Education in 1983.

REFERENCES

1) Roitt, I.M. and Lehner, T. (1980) Immunology of oral diseases, Ist ed. (Blackwell Scientific pub.) p.421, London
2) White, K.L. (1978) ICD 9 CM; The international classification of diseases, 9th Revision, clinical modification vol.1, (Edwards Brothers, Inc., Ann Arbor) p.443, Michigan
3) Davidsohn, I. (1929) Heterophile antibodies in serum sickness, J. Immunol., 16, 259-273
4) Forssman, J. (1911) Die Herstellung hochwertiger spezifischer Schafhaemolysine ohne Verwendung von Schafblut. Biochem. Ztschr., 37, 78-115
5) Paul, J.R. and Bunnell, W.W. (1932) The presence of heterophile antibodies in infectious mononucleosis. Am. J. Med. Sci., 183, 90-104
6) Kasukawa, R., Kano, K., Bloom, M.L., and Milgrom, F. (1976) Heterophile antibodies in pathologic human sera resembling antibodies stimulated by foreign species sera. Clin. Exp. Immunol., 25, 122-132
7) Kumakawa, H., Watanabe, K., Kaise, T., Yoshida, H., and Kasukawa, R. (1981) Characteristics of heterophile antibody with rabbit hemagglutination test. Igaku to Seibutsugaku, 102, 79-83 (in Japanese)
8) Kasukawa, R. (1977) Studies on heterophile antibody of serum sickness type. Igaku no Ayumi, 100, 605-609 (in Japanese)
9) Harada, T. Inokuchi, T., Ikejiri, S., and Uchiyama, C. (1976) Studies on lymphocyte subpopulation in oral diseases, I. Jpn. J. Oral Surg., 22, 443-445 (in Japanese)