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Brief Communication

Passive Hemagglutination for Detection of Autoantibodies against Thyroglobulin in Dogs

Primary hypothyroidism in dogs is mainly caused by autoimmune thyroiditis or idiopathic follicular atrophy of the thyroid gland (*Gosselin et al.* 1982). Serologically, autoimmune thyroiditis can be diagnosed by detection of autoantibodies against thyroid antigens in hypothyroid dogs (*Mizejewski et al.* 1971, *Gosselin et al.* 1980). Passive hemagglutination test (PHT) has been the serological test, most widly used for detection of antibodies (*Mizejewski et al.* 1971, *Gosselin et al.* 1982, *Vollset & Larsen* 1987).

The most time-consuming procedures in connection with the PHT are the preparation of antigens, the collection and preparation of red blood cells and the coating of red blood cells with antigens. Preparation of thyroglobulin coated sheep red blood cells (SRBC) in large amounts and subsequent freezing in several small ampullae may be both time saving and may eliminate errors due to batch to batch variability.

The aim of this study was therefore to establish a PHT using erythrocytes coated with thyroid globulins stored at –196°C.

Serum sampling, preparation and isolation of thyroglobulin and PHT using the chromic chloride method (CCH) and the glutaraldehyde method (GCH), were as described by *Gosselin et al.* (1980), *Avrameas et al.* (1969), *Vollset & Larsen* (1987).

A human hemagglutination kit (Thymune-T^{R}, Wellcome) based on freezedried tanned turkey erythrocytes coated with human thyroglobulin was also tested.

The PHT was performed in microtiter V-bottom plates with two-fold dilutions of test serum and mixed with coated RBC to a final RBC concentration of 0.33 % in all 3 methods used. The PHT results were not influenced when SRBC were collected from different animals. For cryopreservation of thyroglobulin coated SRBC, the cells were suspended in gelatin-veronal-buffered saline-sucrose (GVBSM^{++}-sucrose) and neutralized PVP-10 (Polyvinylpyrrolidone, Sigma) as described by *Myhrvold* (1979).

The cell suspensions were frozen immediately in liquid nitrogen at –196°C in 1.5 ml portions in plastic ampullae (Nunc, Inter Med., Denmark). Thawing of the coated SRBC was carried out at +43°C in a water bath after a 15 s delay with the ampullae at room temperature. After thawing, the cells were washed once in GVBSM^{++}-sucrose and twice in PBS. The cells were then resuspended in PBS to obtain working concentrations of cells coated by the GCH method and by the CCH method of 3.8 % and 0.66 %, respectively. Uncoated SRBC as control were prepared, frozen and thawn in the same way as coated SRBC.

The effect of cryopreservation of coated SRBC was investigated by using freshly coated SRBC and thawed coated SRBC stored at –196°C for 14 and 21 weeks from the same batch in the PHT (Table 1). In the GCH test, the serum titer of thyroglobulin autoantibodies was generally higher for cryopreserved coated SRBC than for fresh

Table 1. Effect of cryopreservation of sheep red blood cells coated with thyroglobulin on the passive hemagglutination titer.

Dog no.	CCH[a] Storage time			GCH[b] Storage time		
	Day 0	14 weeks	21 weeks	Day 0	14 weeks	21 weeks
1*	40	20	20	20	80	80
2	40	40	40	20	320	320
3	160	20	20	20	40	40
4	80	20	0	40	640	640
5	40	0	0	20	320	320
6	20	0	0	20	40	40
7	40	0	0	20	160	160
8	160	0	0	80	0	0
9	20	0	0	0	160	160
10	20	20	20	0	640	640
11	0	n	n	0	n	160
12	0	n	n	0	n	40
13	0	n	n	0	n	640
14	0	n	n	0	n	640
15	20	n	n	20	n	40
16	160	n	n	20	n	40
17	0	n	n	40	n	40
18	80	n	n	40	n	640
19**	0	0	0	0	0	0
20**	0	0	0	0	0	0

[a] CCH – Chromic chloride hemagglutination
[b] GCH – Glutaraldehyde hemagglutination
n not done
0 negative (titer < 20)
* Titer value expressed as inverse titer value
** Healthy dogs

SRBC. The use of frozen SRBC in the GCH test revealed 5 more hypothyroid dogs with thyroglobulin autoantibodies than when fresh SRBC were used in the CCH test (Table 1). A possible explanation may be increased ionic strenght between the red cell bound thyroglobulin and the serum antibodies, a property achieved during storage at –196°C. Increased antibody titers in hemagglutination assay using frozen sensitized SRBC were also observed by *Myhrvold & Akselsen* (1982). Antibody-negative control serum did not agglutinate frozen thyroglobulin coated SRBC and antibodypositive serum did not react with frozen unsensitized SRBC. A general decrease in thyroglobulin antibody titers was observed when frozen coated SRBC were used in the CCH test. This observation may possibly be explained by a change in ionic strength between the metallic cation Cr^{3+} and the erythrocytes due to altered environment caused by the freezing buffer system. This in turn may lead to extensive loss of thyroglobulin from the RBC surface.

The effect on the PHT titer of coated SRBC stored at +4°C was investigated. Serum samples from 6 dogs with thyroglobulin autoantibodies were used both in the CCH and in the GCH test. Storage of SRBC coated by the CCH method was not successful as hemolysis occurred. No effect on the PHT titer was seen when SRBC coated by the GCH methods were stored at +4°C for 20 days. No agglutination in the PHT was detected when Thymune-T^R kit was used for detection of canine thyroglobulin antibodies. This study showed that thyroglobulin coated SRBC can be stored at –196°C for at least 21 weeks by using the GCH method. SRBC prepared by the CCH method had to be used in the PHT immediately after thawing, since a progressive hemolysis was observed soon after thawing, being complete after storage at +4°C for 10 days. We therefore recommend frozen (–196°C) SRBC prepared by the GCH method for use in the PHT to assay autoantibodies against thyroglobulin in dogs.

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