

Complement-dependent Cytotoxic Crossmatch for Prevention of Hyperacute Rejection in Canine Renal Allografts

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Abstract: DLA class I complement-dependent cytotoxicity (CDC) cross-match method was established to control hyperacute rejections in organ transplantation. The aim of the present study is to investigate if DLA class I CDC cross-match method is effective to prevent hyperacute rejections in canine renal allografts. Seven mongrel dogs of similar age and weight were used. Erythrocyte crossmatch was first performed and only the negatives were used. Among the same blood types, CDC cross-match was performed. Anti-dog serum, Hank's balanced salt solution (HBSS), and the self-serum was used as a positive-, a negative-, and an auto-control respectively. After the reaction with class I complement, it was stained with eosin and scored by international cytotoxicity scoring system under inverted phase microscope. According to these results, kidneys of CDC negatives among same blood types were cross-transplanted to observe the incidence of hyperacute rejections. One of four 1.2 B blood type dogs had autoantibodies. here were negative CDC results among each blood type, and also there were negative results between different blood types. Two pairs with the same blood types and negative CDC results underwent allo-transplantation each other. There were no hyperacute rejections. DLA cross-match method studied in this experiment for canine renal allograft can be effective to prevent hyperacute rejections. It may be applicable for the future studies of histocompatibility testing in canine renal allografts.

Key words : kidney, allograft, hyperacute rejection, lymphocytes, cytotoxicity.

Introduction

HLA crossmatch significantly enhance survival of renal transplants³. In this study, RBC type was determined and cytotoxicity test based on DLA (dog leukocyte antigen) class I was experimented to survey the outcome of the renal transplant.

The antigen coded from MHC (major histocompatibility complex) is exposed on the surface of the cells, which is the key to the self-recognition. MHC antigen expressed in the dog leukocyte is called DLA, dog leukocyte antigen¹³. Three groups of histocompatibility antigens are of major importance in stimulating rejection. These are the MHC class I, class II molecules and the major blood group glycoproteins¹⁹.

Class I antigens originate from serologically defined (SD) loci of MHC, and are expressed on almost all cell surfaces with exception of mature erythrocytes. There are three SD loci in the dog, and are termed DLA-A, B, C. These antigens are responsible for inducing humoral antibodies¹³.

Class II antigens are coded from lymphocytes-defined (LD) loci, also known as lymphocytes activating determinants (LAD) loci. LD antigens are restricted to B cells, antigen-presenting cells (APCs) such as dendritic cells and macrophages. These antigens induce T cell activation, but do not easily induce antibody production¹³.

Blood group antigens are primarily B cell stimulator. Released by the cells of the allograft, such as blood transfusion, these antigens trigger B cell responses and antibody formation. Dogs can possess preexisting natural antibodies to some of these antigens, they can trigger a hyperacute rejection response¹⁹.

In human, routinely diagnosed clinical laboratory tests for renal transplant include ABO blood typing, HLA tissue typing, and crossmatching. Natural IgM antibodies specific for allogenic ABO blood group antigens will cause hyperacute rejection¹. HLA typing or assay refers to a method of determining an individual's HLA-ABC, or DR/DQ type using a ready-made panel of well-characterized antisera of defined HLA specificities⁶. The crossmatch will determine whether the patient has antibodies that react specifically to the potential donor cells. It is similar to the typing, but in this case, the recipient's serum is tested for reactivity against only the donor's lymphocytes. Cytotoxic and flow cytometric assay can be used. Only IgG and IgM are responsible for complement activation, since only these antibodies have complement binding sites¹.

The crossmatch is based on complement-dependent cell cytotoxicity (CDCC). It is also known as complement-dependent microcytotoxicity (CDMC), micro-lymphocytotoxicity, or lymphocytotoxicity crossmatch. The crossmatch detects the presence of donor cell specific antibody in recipient's peripheral blood. However, bound antibody alone can not produce any recognizable results other than neutralization and optimization, *in vitro*. Complement is introduced to induce cell lysis. For class I typing a mixed

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lymphocytes population can be used, whereas for class II typing a pure B lymphocytes preparation is essential⁷. In this study, only class I crossmatch was experimented.

A positive cytotoxic crossmatch against donor T cells is usually considered to be an absolute contraindication to transplantation with the specific donor. If a recipient has strong cytotoxic antibody against histocompatibility antigens expressed on donor cells at the time of engraftment with a kidney, the damage will be immediate. This type of hyperacute rejection is largely be avoided by pretransplant crossmatching of recipient serum with lymphatic cells from the donor⁷.

The aim of the present study is to investigate if DLA cross-match method is effective to prevent hyperacute rejections in canine models.

Materials and Methods

Total eight mongrel dogs were used in this experiment. Seven male dogs (dog 2-8) and one female dog (dog 1) were used. Most dogs were about a year old, weighing about five kilograms. Major and minor red blood cell crossmatch was performed prior to blood typing. Candidates for donors and recipients were selected based on erythrocyte typing. Dog erythrocyte antigen was determined using commercial canine blood typing kit (Canine blood typing kit, Shigeta, Japan). Dog leukocyte antigen was done as below.

Sampling

Two kinds of blood samples are needed from each candidates: Heparinized whole blood samples for peripheral blood mononuclear cell isolation, and serum samples for antibody. The dosage of heparin is 10 U per ml of blood⁵. In this study, 10 ml of heparinized donor whole blood samples, and 2 ml of blood samples in plain tubes were collected. Process of heparinized whole blood should yield 10^6 PMBCs per 10 ml⁵, hence the volume can be adjustable to the protocol, the desirable cell concentration, or the fluency of the isolation technique. The blood samples should be kept at room temperature to keep cell viability⁵.

PBMC isolation

The dilution of the whole blood allows effective isolation of PBMC⁵. Each heparinized whole blood were transferred to 50 ml conical centrifuge tubes. Equivalent volume of Ca^{++} and Mg^{++} free Hank's balanced salt solution (HBSS, Gibco) was mixed with the sample prior to the isolation process⁸. It is advisable to add a few drops of antibiotic to HBSS, such as penicillin-streptomycin. PBMC can be isolated from whole blood using lymphocytes separation medium, Ficoll solution (Histopaque-1077, Sigma). The density of Ficoll solution is 1.077 g/ml at room temperature, which allows separation of mononuclear cells from granulocytes and erythrocytes during centrifugation⁷.

This is done by carefully laying 2 volumes of 1:1 mixture of heparinized whole blood and HBSS, over 1 volume of Ficoll solution in 15 ml conical centrifuge tubes⁸. The process should not disturb the surface of Ficoll solution, or else much RBC contamination will be likely.

Centrifuge at 1800 rpm for 20 minutes at room temperature. PMBC is separated through a density gradient as a distinct band between the plasma and the Ficoll solution. Aspirate the PMBC layer carefully using plastic transfer pipette, not to disturb the erythrocytes below the Ficoll layer. Resuspend the isolated PMBC with two-fold volumes of HBSS in 15 ml conical centrifuge tube, and mix vigorously with the transfer pipette. Centrifuge at 1200 rpm for 10 minutes at RT. Discard the supernatant and repeat the washing procedure. Centrifuge at 800 rpm for 10 minutes at RT. Discard the supernatant. This last procedure of centrifugation at low speed will eliminate much of the thrombocytes in plasma. The platelets express class I antigens, and their presence reduces the amount of antibody available for lymphocytes⁵, which may lead to false negative results. It is recommended to do the washing procedure at least twice to eliminate all remaining platelets⁷.

The pellet is then suspended with 10 ml of 10% heat-inactivated fetal calf serum (Gibco) in HBSS. This method should yield 10^6 PMBCs per 10 ml of blood processed⁵.

Cell count

For complement-dependent cytotoxicity test, viable cell counting procedure is essential, because the test is based on comparison of living to dead cells. The counting can be done manually by using hemocytometer. Trypan blue 0.4% is used to dye the dead cells only, and viable cells remain round and translucent. Equal volume of the dye and the sample is mixed using micro pipette. The mixture is introduced under the cover-slip and the chamber fills by capillary action. Allow lymphocytes to settle on a microscope, and count the unstained mononuclear cells. Dead cells are stained blue, and erythrocytes are pinkish. Viable cells exclude the dye and remain refractile².

Calculate the original viable cell concentration by multiplying the dilution factor. Thus, The number of cells counted $\times 2$ (dilution by equal volume of the dye) $\times 10^4$ = cells/ml of suspension⁹.

Cell concentration of 2×10^6 cells/ml is required for the test^{5,7,8}. Adjust the cell concentration by centrifuging and leaving only right amount of the medium to resuspend. For example, if the calculated cell concentration is 1×10^6 cells/ml, centrifuge at 1000 rpm for 10 minutes, and discard only top 5 ml of the supernatant leaving 5 ml undisturbed with the pellet. Resuspended pellet is two-fold concentrated.

The crossmatch plate preparation

Plain blood samples from each candidates in 2 ml ependorf tubes are sit for at least 30 minutes, during the isolation procedure. Coagulation will separate the antibody within

the serum from the cellular component. Centrifuge at 3000 rpm for 20 minutes. Transfer the clean supernatant to a new endpoff tube. Serum can be depleted of complement activity by heating it to 56°C for 30 minutes¹⁹. But in many laboratories, it is omitted since the effect is negligible.

Panspecific DLA antiserum is used as a positive control serum⁵. The sera can be obtained from bitches in their second or subsequent pregnancy shortly after birth. About 10-20% of sera tested will contain specific antibodies but only 10% will prove to be useful⁷. Therefore sera from many different parturated bitches can be collected to obtain a source of panspecific antisera. But this whole process can be troublesome. Cytotoxic monoclonal antibody directed against HLA class I (w6/32) and class II (L243) are available, and routinely used as positive controls in the cytotoxicity assay⁷. In this study, anti-dog serum from rabbit (Anti-dog whole serum developed in rabbit, Sigma) was used as a positive control serum. It was heated to 56°C for 30 minutes to inactivate the complements. For a negative-control serum, 10% FCS (Heat-inactivated fetal calf serum, Gibco) in HBSS (Hanks's balanced salt solution, Gibco) was used.

Use a Hamilton syringe to shoot 1 µl of antisera in each 72-well Terasaki typing plates as designated (Table 1). After dispensing the serum, layer 5 µl of light mineral oil to prevent from drying off.

The lymphocytotoxicity crossmatch

Shoot 1 µl of cells to each well of the Terasaki plates through mineral oil using a Hamilton syringe. For instance, cells from dog X are shot to row 1, 3, 6, 7, 9, 12 and cells from dog Y are shot to row 2, 4, 5, 8, 10, 11. Ensure that the cells are well mixed with serum under oil in each wells⁵. Incubate at room temperature for 30 minutes for row 1 through 6, and 60 minutes for the rest.

Add 5 µl of freshly thawed HLA class I rabbit complement (Class I complement, One Lambda) to each well^{5,8}. Rabbit is the most effective source of complement for use in the micro lymphocytotoxicity tests⁷. Ensure that it is well mixed and incubate at room temperature for 30 minutes for row 1-6, and 120 minutes for row 7-12.

Add 2 µl of 5% eosin Y to each well⁸. Eosin solution should be filtered and stored in refrigerator¹⁸. Because swelling of the dead cells is increased by addition of eosin, it is important to allow the dye to penetrate for two minutes¹⁸.

After 2 minutes, add 5 µl of buffered 37% formalin to

each well. The formalin should be buffered to pH 7.4 with NaOH, and filtered⁸.

Inverted phase contrast microscope on 100 magnification was used to read the results on Terasaki plates. Entire reaction can be seen in one field without examining different areas¹⁸. Living cells are small and retractible, whereas dead cells appear larger, non-retractible, and darker⁸. The percentage of cell death in each well was rated according to International Histocompatibility Workshop (IHW) cytotoxicity scoring system^{7,11}. The percentage of cell lysis 0-10% is scored '1' being negative, 11-20% as '2' being probable negative, 21-50% as '4' being weak positive, 51-80% as '6' being positive, 81-100% as '8' being strong positive, whereas '0' represents not readable or invalid. The cytotoxic scores are rated in comparison to controls in each individual tests.

First four rows are the control panels, and the last two rows are the actual crossmatch tests (Table 1). Positive controls are for the assurance of active complement. Negative controls are used to determine the viability of the donor cell preparation. It is used in comparison with the recipient's serum to evaluate the crossmatch results. The third and fourth rows are the autocontrols to determine whether autoantibodies directed against its own cells are present in its serum. It may cause a false-positive reactions, since autoantibodies usually react nonspecifically with all lymphocytes. The fifth and sixth rows are the crossmatch test between donor cells and recipient serum.

Scoring

The dead cells were counted by dividing the photos of a well into sixteen parts, and four out of sixteen parts were counted. The negative control in each test of each incubation time was compared relatively to calculate the percentages. Scores were then graded according to the cytotoxicity scoring system of the International Histocompatibility Workshop. A 20% increase in the percentage of cell deaths was considered a positive crossmatch⁶.

Results

The major and minor RBC crossmatch were performed among eight dogs. One dog was excluded from the candidate due to positive crossmatch with all. Rest of the seven dogs' blood type was determined using commercial canine

Table 1. Preparation of serum panel of a recipient (R) and a donor (D) in Terasaki typing plate

	A	B	C	D	E	F
1	(+)control	(+)control	(+)control	(-)control	(-)control	(-)control
2	(+)control	(+)control	(+)control	(-)control	(-)control	(-)control
3	R	R	R	R	R	R
4	D	D	D	D	D	D
5	R	R	R	R	R	R
6	D	D	D	D	D	D

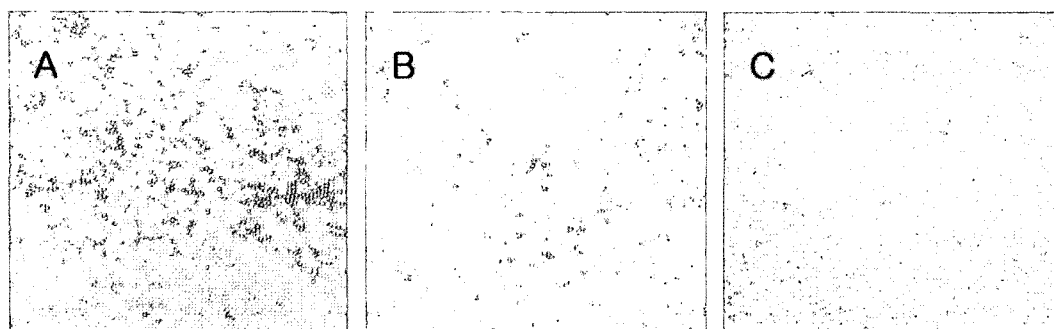


Fig 1. The photograph showing cytototoxicity score under inverted phase contrast microscope (x100, eosin stain). In grade 1, cell lysis less than 10%, retractible and viable cells are seen throughout the field. Up to grade 2, cell death less than 20% are considered a negative (A). In grade 4, up to 50% cells die, which is considered a weak positive, but nevertheless positive. Grade 6 means more than 80% cell deaths, and cells are scarcely seen (B). Grade 8 is a strong positive, and should be the result of the positive control. Eosin stained lysed cells appear through out the clear field like the shadow of the cells (C).

Table 2. The result of lymphocytes cross match in dog 3 showing the presence of autoantibodies

Recipient ID (blood type)	Donor ID (blood type)	Cytotoxic score	
		30 min	60 min
3 (1.2B)	3 (1.2B)	8 (+)	8 (+)
2 (1.2B)	3 (1.2B)	8 (+)	8 (+)
3 (1.2B)	2 (1.2B)	8 (+)	8 (+)

blood typing kit (Shigeta, Japan). Four dogs (dog 2, 3, 4, 6) were Shigeta 1.2 B, and three dogs (dog 1, 5, 7) were Shigeta 1-negative B. The lymphocytes crossmatch were performed among the dogs with same blood types.

On autocontrol wells, none but dog 3 had positive results in crossmatch with dog 2 (Table 2). Crossmatch was also strong positive. Hence, dog 3 was excluded from the candidate.

The lymphocytes crossmatch was done among same blood types to sort out the best matching pairs to cross allograft the kidney. Pairs with negative results such as grade 1 or 2 on 30 minutes incubation period was selected (Table 3). The results of dog 6 and dog 7 lymphocytes crossmatch was also negative.

Cross renal allografts were performed between selected pairs based on negative results of 30 minutes incubation period. Post-surgery immunosuppression were maintained using cyclosporine, prednisolone, and azathioprine. Of the pairs with same blood type, hyperacute rejection did not occur which matches with the negative results of the 30 minutes incubation period. However, dog 4 manifested acute rejection at day 19 and was euthanized.

Discussion

Blood group antigens trigger the antibody formation by B cells¹⁹. ABO blood typing is critical in human to avoid hyperacute rejection because of natural IgM antibodies¹. ABO antigens are expressed on many other cell types in addition to erythrocytes, including endothelial cells¹.

Dog Erythrocyte antigen is mainly classified as 1, 3, 4, 5, and 7⁴. DEA 1 has subgroups 1.1 and 1.2^{4,14}. The significance of the DEA 7 antigen is under debate, and many dogs are not tested against for this antigen¹². In dogs, only DEA 1 is strong enough to be of clinical importance, which is also known as 'A' alloantigen¹⁹. About 60% of dogs has been known to be DEA 1 positive^{17,19}. Naturally occurring antibodies to DEA 1 occur in about 10% of DEA 1 negative

Table 3. The results of hyperacute rejection associated with the blood type and the cytotoxic score

Blood Type*	Recipient ID	Donor ID	incubation times(min)				Hyperacute Rejection
			30		60		
			Cell death (%)	Score	Cell death (%)	Score	
1-B	1	5	9.2±3.2	1	14.4±10.9	2	No
	5	1	3.5±0.3	1	7.2±2.7	1	No
1.2B	2	4	18.6±5.5	2	25.1±12.4	4	No
	4	2	17.6±12.0	2	26.4±11.0	4	No
Mixed	6	7	9.2±5.4	1	16.8±13.4	2	No
	7	6	16.5±7.7	2	27.4±13.3	4	Died**

*Shigeta canine blood typing, **Died due to anesthetic accident at surgery

dogs, but are usually of low titer and not of clinical importance¹⁹. Therefore, dogs are commonly said to have no naturally acquired DEA I antibodies^{11,13}. This is the reason why unmatched first transfusions are usually safe in dogs¹⁹.

The commercial kit used in this experiment is composed of the monoclonal antibody against DEA 1.1, DEA 1.2, Shigeta A, and Shigeta B. Shigeta A corresponds DEA 3 and 5, and Shigeta B corresponds to DEA 4 and 6. The blood type of dog 2, 3, 4, 6 was 1.2 B which means they possess DEA 1.2, and, DEA 4 or 6 antigens. The blood type of dog 1, 5, 7 was 1-negative B which means they do not have DEA I antigen and they have DEA 4 or 6 antigens.

Rejection due to matured Erythrocyte can be critical in blood transfusion since most of the cells constituting the blood are the red blood cells. However, in DLA crossmatch results, only mononuclear cells are mixed with potential donor's serum. The source of Erythrocyte antigen is not present at the DLA crossmatch even if there exist antibodies specific to donor's Erythrocyte. And, in this experiment the possibility of antibody against DEA was eliminated earlier by red blood cell crossmatch.

Contamination with Erythrocyte, platelets, or granulocytes can produce non-reproducible results¹⁸. Erythrocyte contamination should be minimized during PBMC procedure. Excessive numbers of Erythrocyte can consume complements¹⁸. If too many red blood cells are seen at the cell counting level, restarting the experiment is recommended. When stained with trypan blue, Erythrocyte appear smaller and brownish, compared to larger and translucent lymphocytes.

In last procedure of washing, centrifugation at low speed will eliminate much of the thrombocytes in plasma. The platelets express class I antigens, and their presence reduces the amount of antibody available for lymphocytes⁵. Granulocytes often produce false-positive results by being more sensitive to the cytotoxic effects of the complement, or sticking to the bottom of the tray¹⁸. Under phase contrast microscope, these sticking cells appear as dark, dead cells, making it very difficult to distinguish between the dead granulocytes and the dead lymphocytes¹⁸.

Insufficient numbers of cell will produce false-positive reactions and excessive numbers will produce false-negative reactions¹⁸. I should admit that in this study as much as twice the desired cell concentration of 2×10^6 cells/ml was used. Because the percentage of cell lysis was calculated in comparison to each negative control, this variation in cell concentration might have lowered the cell death percentage by relative shortage of the antibody.

Prolonging the incubation time was done with extra 30 minutes comparing to the standard NIH method. Either the short or the long procedure for class I, 30 minutes incubation is recommended for the cell and the serum reaction⁶. It is suggested that the sensitivity of the lymphocytotoxic crossmatch can be enhanced by either prolonging the incubation time¹⁰.

Further, B cell isolation can be added to this method for

DLA class II evaluation. This procedure was traditionally done by rosetting T cells with sheep Erythrocyte and removing them by density centrifugation gradient⁷. Or nylon wool columns can be used⁷. B cells adhere to the nylon wool during incubation period at 37°C and can be retrieved by flushing the column with cold media⁶. But this procedure may require some technical achievements. Moreover it is time-consuming and B cells can be contaminated with T cells. Today, the most reliable technique for the pure B cell isolation is by using immunomagnetic beads⁷. It is faster and highly purified populations of B cells can be obtained⁶.

In this study, 10 ml of heparinized whole blood was taken from each dog for DLA class I crossmatch since it should yield 10^6 PBMCs per 10 ml⁵. For class II crossmatch, usually requires 20 ml of heparinized blood samples if B cells are isolated with nylon wool columns. For small dogs and especially for toy breeds this amount of samples can be risky. Moreover, class II rabbit complements should be used for the B cells.

Only class I crossmatch was performed in this experiment and it can be assumed that class II could have been the factor for the different outcome. Of 4136 human cadaver kidney transplantation, using ELISA for IgG-anti-class I and class II antibodies, good 2-year graft survival rate was $85 \pm 3\%$ for HLA class I-positive/ class II-negative, $84 \pm 2\%$ for HLA class I-negative/ class II-positive, and $71 \pm 3\%$ for HLA class I-positive/ class II-positive¹⁶. According to the study, negative response from either both or one of the class resulted in similar survival rates, and only those with antibodies positive to both class I and class II had a significantly lower graft survival rate. This could be interpreted as class I negatives can be suitable for kidney transplant on no consequences of class II compatibility. Only the existence of the antibody against both classes at the time of transplantation signal an increased alloreactivity threshold at which current immunosuppressive protocols tend to fail¹⁶. In other study, the presence of HLA class I antibodies in the crossmatch sample were significantly associated with lower graft survival at one year compared with patients without donor-reactive antibodies¹⁵.

In this preliminary study, two pairs of the same blood types and negative CDC results were transplanted each other. There were no hyperacute rejections.

The results of the present study support that DLA crossmatch method studied in this experiment renal allografts can be effective to prevent hyperacute rejections.

Conclusions

In this preliminary study, two pairs of the same blood types and negative CDC results were transplanted each other. There were no hyperacute rejections.

DLA cross-match method studied in this experiment renal allografts can be effective to prevent hyperacute rejections. It is hope to be applicable for the future studies

and practices of histocompatibility testing in canine renal allografts.

Acknowledgement

This study was supported by 2004 Kangwon National University Grant. Many gratitude to professor Kwon HM, Han TW, Park SI, and Dr. Park YJ. Kim MK and Kang MS for countless laboratory supports and advice. Many thanks to Institute of Animal Resources Kangwon National University. This work was partially supported by SHIGETA Animal Pharmaceuticals Inc.

References

1. Abbas AK, Lichtman AH. Cellular and molecular Immunology. 5th ed. Philadelphia: Saunders. 2003; 48, 384-386
2. Chapel H, Sewell H. Cellular assays for lymphatic immune deficiency. In: Clinical Immunology: a practical approach. Oxford: Oxford University Press. 1990: 84-87.
3. Dyer PA, Johnson RWG, Martin S, et al. Evidence that matching for HLA antigens significantly increases transplant survival in 1001 renal transplants performed in the northwest region of England. Transplantation 1989; 48: 131-135.
4. Hale AS. Canine blood groups and their importance in veterinary transfusion medicine. Vet Clin North Am Small Anim Pract 1995; 25: 1323-32.
5. Krausa P, Browning M. HLA typing methods. In: Lymphocytes, a practical approach. Oxford: Oxford University Press. 2000: 230-239.
6. Lou CD, Cunniffe KJ, Garovoy MR. Histocompatibility testing by immunologic methods: humoral assays. In: Manual of Clinical Laboratory Immunology, 5th ed. American Society for Microbiology. 1997: 1087-9, 1093-6.
7. Martin S, Dyer PA. Identification and importance of MHC, the definition of HLA specificities by cytotoxicity. Transplant Immunology 1994; 2: 108-115.
8. Miller LE et al. Manual of Laboratory Immunology. 2nd ed. Lea & Febiger. 1991: 84-86, 412-413.
9. Plebanski M. Preparation of lymphocytes. In: Lymphocytes, a practical approach. Oxford: Oxford University Press. 2000: 6-7.
10. Ross J, Dickerson T, Perkins HA. Two techniques to make the lymphocytotoxic crossmatch more sensitive: prolonged incubation and the antiglobulin test. Tissue Antigens 1975; 6: 129-36.
11. Schlenke P, Kluter H, Muller-steinhardt, Hammers HJ, Borchert K, Bein G. Evaluation of a novel mononuclear cell isolation procedure for serological HLA typing. Clinical and diagnostic laboratory immunology 1998; 5: 808-813.
12. Smith CA. Transfusion medicine: the challenge of practical use. J Am Vet Med Assoc 1991; 198: 747-752.
13. Stevenson S, Schwartz A. Transplantation Immunology. In: Textbook of Small Animal Surgery. Philadelphia: WB Saunders. 1985: 199-212.
14. Stone E, Badner D, Cotter S. Trends in transfusion medicine in dogs at a veterinary school clinic: 315 cases (1986-1989). J Am Vet Med Assoc 1992; 200: 1000-1004.
15. Sumitran-Holgersson S. HLA-specific alloantibodies and renal graft outcome. Nephrol Dial Transplant 2001; 16: 897-904.
16. Susal C, Opelz G. Kidney graft failure and presensitization against HLA class I and class II antigens. Transplantation 2002; 73: 1269-1273.
17. Swisher SN, Young LE. The blood grouping systems of dogs. Physiol Rev 1961; 41: 495-520.
18. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet Testing for HLA-A, B, C, and D Antigens. Am J Clin Pathol 1978; 69: 103-120.
19. Tizard. Veterinary Immunology, An introduction. 5th ed. Philadelphia: WB Saunders. 1996: 216, 365-394.

개의 신장 동종 이식에서 초급성 거부반응의 예방을 위한 보체의존성 세포독성 DLA 클래스 I 교차 반응

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요약 : 보체의존성 세포독성반응(CDC)을 이용한 DLA class I 교차 반응의 실험방법을 정립함으로써 개의 동종 신장 이식 후 초기에 발생하는 초급성 거부반응을 억제하는데 응용하고자 본 실험을 실시하였다. 체중(약 5 kg)과 연령(약 1년령)이 유사한 짝종견을 대상으로 적혈구 교차 반응을 실시하여 상호 음성인 7마리를 실험에 사용하였다. 혈액형이 동일한 개체를 대상으로 CDC검사를 실시하였으며, Anti-dog serum, Hank's balanced salt solution (HBSS), 그리고 자가 혈청을 각각 양성, 음성, 그리고 자가 대조 혈청으로 이용하였다. Class I 보체와 반응시킨 후 에오신으로 염색하여 고정된 다음 위상차 현미경 100배율에서 조사하였다. 국제 Cytotoxicity scoring system에 의하여 죽은 세포가 20% 이상이면 양성으로 평가하였다. CDC 결과 동일 혈액형 군에서 상호 음성이 나온 경우를 대상으로 상호 동종이식을 실시하여 초급성 거부반응의 발생 정도를 평가하였다. 혈액형이 1.2 B인 4두 중 1두는 자가항체를 가지고 있었다. CDC 결과 동일 혈액형 군에서 각각 1쌍이 상호 음성을 나타내었고, 혈액형이 다른 1쌍에서도 상호 음성이 관찰되었다. 혈액형이 동일하고 CDC 음성인 2쌍 4두를 대상으로 상호 신장 이식을 한 결과 4마리 모두 초급성 거부반응이 나타나지 않았다. 이 실험에서 확립한 DLA 교차 방법은 동종 이식에서 초급성 거부반응을 억제하는데 효과적인 방법이며, 향후 개의 동종 장기 이식에서 조직적합성 평가를 위해 응용될 수 있을 것이라 사료된다.

주요어 : 신장, 동종이식, 초급성 거부반응, 임파구, 세포독성