The degree of major histocompatibility complex matching between purebred Maltese and mongrel dogs using microsatellite markers

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ABSTRACT

Long-term maintenance of transplanted organs is one of the major factors that increases survival time of recipients. Although obtaining a major histocompatibility complex (MHC)-matched donor with the recipient is essential for successful organ transplantation, there have been limited reports on MHC matching between dogs. In this study, we analyzed the canine MHC matching rates using Maltese, one of the most popular purebred dogs, and mongrel dogs in Korea. Genomic DNA was extracted from blood leukocytes and DNA was amplified by polymerase chain reaction with primers specific to MHC microsatellite markers. The MHC matching degree was confirmed by the microsatellite markers using polyacrylamide gel electrophoresis. The MHC matching rates of each donor-recipient groups including Maltese-Maltese, mongrel-mongrel and Maltese-mongrel were 4.76%, 5.13% and 6.67%, respectively. There were no significant differences in the MHC matching degree between each group. These results demonstrate that MHC-matched donors could be selected from other breeds as much as from the same breed for transplantation. Knowledge of the MHC matching degree of purebred and mongrel dogs would offer valuable information not only for improving the success rate of organ transplantation surgery in canine patients but also for transplantation research using experimental canine models.

Keywords: Microsatellite markers; major histocompatibility complex; dogs

INTRODUCTION

Organ transplantation has become an accepted medical treatment for canine patients with end-stage organ failure [1-3]. However, survival after kidney transplantation in canine has been reported to be as low as 36% after 100-days, unlike in human clinical cases, wherein the 1-year patient survival rate is over 90% [2,4]. One of the major reasons leading to these unfavorable results in dogs might be insufficient pre-surgical tests that only screen for hyperacute rejection but not acute or chronic rejection caused by major histocompatibility complex (MHC) incompatibility [2,3,5,6]. Experimentally, the survival rate of canine kidney transplantation in MHC-identical or haploidentical groups was significantly higher, at more than 4 years, with immunosuppressants [7,8]. In general, the same canine breed is required as the organ transplant donor to prevent rejection. However, there is no data comparing
MHC matching between different breeds. Therefore, such data would be beneficial for canine kidney transplantation.

Moreover, canines are widely used as one of the reliable preclinical, large-animal models for organ transplantation and immunological research [7,9]. Although small animal models, including mice and rat, incur lower costs to acquire and maintain, there are several limitations of using small animals in transplantation research [10,11]. Canines have strong similarities in surgical anatomy, physiology, and surgical techniques with humans and have contributed to the development of transplantation medicine fields [12,13].

Disparities in various polymorphic systems, mainly MHC, are the most important factors determining immunological rejection of transplants [14-16]. The canine MHC, or dog leukocyte antigen (DLA) complex, is poorly characterized, with only eight functional genes and five pseudogenes identified to date for the class I and II MHC gene region [17]. Furthermore, canine specific monoclonal antibodies for serological typing are not available and sequence-based typing methods of MHC alleles are time consuming [18]. Analysis of polymorphic microsatellites or short tandem repeats (STR) spanning the MHC provides an alternative method for rapid and accurate characterization of the region [19-21]. However, research on the canine microsatellite marker related to DLA have been limited to specific breeds [22].

The knowledge about MHC matching between different canine breeds would not only contribute to increasing the survival rate of transplantation in clinics but also would be helpful for studies using canine models. The aim of this study was to compare the diversity of MHC matching from purebred and mixed breed dogs using highly polymorphic microsatellite markers.

MATERIALS AND METHODS

Sample collection and DNA extraction
A total of 28 canine blood samples, which have the DEA 1.1 positive blood type, were obtained from the veterinary medical teaching hospital in Korea in micro EDTA tubes and stored at −20°C until DNA isolation. The samples were composed of two canine species, including 15 Malteses and 13 mongrels. DNA was isolated from leukocytes using the DNA extraction kit (Intron, Sungnam, Korea). The concentration of the DNA samples was measured using a spectrophotometer (Nanodrop 2000c; Thermo Scientific, Waltham, MA, USA).

Polymerase chain reaction (PCR) and DNA electrophoresis
The primer sequences of microsatellite markers of DLA class I and II, FH2200 and FH2202, are listed in Table 1, according to previous reports. PCR was performed using PCR master mix (2X TOPsample™ DyeMIX; Enzynomics, Daejon, Korea) with genomic DNA, specific primers, and distilled water. PCR amplification was performed using the T Professional standard 96 gradient machine (Biometra, Goettingen, Germany) with the following conditions: denaturation at 94°C for 3 min, followed by 39 cycles at 94°C for 30 sec, 59°C for 45 sec and 72 °C for 30 sec. A final extension step was followed at 72°C for 10 min. Trisacetate-EDTA polyacrylamide gel (4.5%) electrophoresis was used to detect the PCR products. PCR products were loaded into wells using electrophoresis equipment (EPS 2A200; Amersham Biosciences, Little Chalfont, UK) for 5 h (FH200) and 3 h 50 min (FH2202) under
100 voltage. One or 2 bands of DLA were shown by autoradiography with UV light (Gbox EF2; Syngene, Cambridge, UK).

**DLA microsatellite analysis**

The visualized bands of MHC class I or II of each dog were designated by alphabet letters according to their band size, as described previously ([Supplementary Figs. 1 and 2](#supplementary)) [22,23]. We assigned the results into 3 groups according to the matching degree; full-match, haplo-match, and unmatched groups. Donor-recipient pairs were divided into 3 groups; Maltese-Maltese, mongrel-mongrel, and Maltese-mongrel.

**Statistical analysis**

Fisher’s exact test was used for statistical analysis. Difference was considered significant at \( p < 0.05 \).

**RESULTS**

A total of 105, 78, and 195 donor-recipient pairs were used, comprising Maltese-Maltese, mongrel-mongrel, and Maltese-mongrel dogs, respectively. The percentage of MHC class I matching from Maltese-Maltese, mongrel-mongrel, and Maltese-mongrel are shown in **Table 2**. The degree of MHC class I full-match was 8.57%, haplo-match was 10.48%, and unmatched was 80.95% in the Maltese-Maltese pair. The degree of MHC class I full-match was 0%, MHC class I haplo-match was 21.79%, and MHC class I unmatched was 78.21% in the Maltese-mongrel pair. The degree of MHC class I full-match was 3.59%, MHC class I haplo-match was 16.41%, and MHC class I unmatched was 80.00% in the mongrel-mongrel pair. The results do not show significant differences in MHC class I matching from each donor-recipient pair group.

The percentage of MHC class II matching from Maltese-Maltese, mongrel-mongrel, and Maltese-mongrel are shown in **Table 3**. The degree of MHC class II full-match was 0.95%, haplo-match was 19.05%, and unmatched was 80.00% of in the Maltese-Maltese pair. The degree of MHC class II full-match was 0%, haplo-match was 21.79%, and unmatched was 80.00%.

### Table 1. The sequence of primer of DLA microsatellite marker

| Primer | Sequence |
|--------|----------|
| MHC class I FH2200 | Forward 5’-GGCATGATCGTGGAGTCCC-3’<br>Reverse 5’-CCCACCCCAGTTGTCCTATT-3’ |
| MHC class II FH2202 | Forward 5’-GTTGAGTGGTTGCCTTTAGC-3’<br>Reverse 5’-CAGGATCTTCATATGTCACC-3’ |

DLA, dog leukocyte antigen; MHC, major histocompatibility complex.

### Table 2. The degree of MHC class I in donor-recipient pairs from Maltese and mongrel dogs

| Group* | No. of full match pairs (%) | No. of haplo-match pairs (%) | No. of nonmatch pairs (%) | Total |
|--------|-----------------------------|-----------------------------|---------------------------|-------|
| Maltese-Maltese | 9 (8.57) | 11 (10.48) | 85 (80.95) | 105 (100.00) |
| Mongrel-Mongrel | 0 (0) | 17 (21.79) | 61 (78.21) | 78 (100.00) |
| Maltese-Mongrel | 7 (3.59) | 32 (16.41) | 156 (80.00) | 195 (100.00) |
| Mongrel-Mongrel | 7 (3.59) | 32 (16.41) | 156 (80.00) | 195 (100.00) |

*Pair of donor-recipient.

There were no significant differences between all groups \( (p > 0.05) \).

MHC, major histocompatibility complex.

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78.21% in the Maltese-mongrel pair. The degree of MHC class II full-match was 2.56%, haplo-match was 25.13%, and unmatched was 80.00% in the mongrel-mongrel pair. The results did not show significant differences in MHC class II matching from each donor-recipient pair group.

Overall, the degree of MHC matching in class I and class II from Maltese-Maltese, mongrel-mongrel, and Maltese- mongrel are shown in Table 4. The number of MHC matching pairs were 5 (4.76%), 4 (5.13%), and 13 (6.67%) in Maltese-Maltese, mongrel-mongrel, and Maltese- mongrel groups, respectively. The results do not show significant differences in MHC matching from each donor-recipient pair group.

DISCUSSION

Microsatellites or STRs are di-, tri-, or tetra nucleotide repeats showing sufficient length variation in the alleles [2,24]. Two polymorphic microsatellite markers, tetranucleotide repeats of (GAAA)<sub>n</sub> or (GATA)<sub>n</sub>, have been reported in dogs; one is C.2200, which is located in the MHC class I region near DLA-53, and the other one is C.2202, which is located in the MHC class II region near DLA-DRB2 [25].

Analysis of MHC matching between different canine breeds is necessary because it is difficult to find blood-related organ donors in companion dogs compared to that in humans. In addition, dogs have a higher transplant failure rate than human and feline recipients because of less effective immunosuppressants to control rejection; however, the reasons are not well-defined yet. Although kidney transplantation has usually been performed without MHC matching in feline patients due to difficulties in obtaining a transplantable organ from blood related donors similar to dogs, post-op prognosis is better than canine recipients [2,26]. Based on previous literature, the median survival time of kidney transplant recipients from unrelated donors have ranged from 360 to 613 days in feline [26]. In contrast, a previous report shows that the median survival time after kidney transplant was 24 days in canine recipients from unrelated donors [2].
Non-MHC proteins derived from different canine breeds could also induce chronic rejection [27]. However, studies on kidney transplantation using MHC-matched mongrel dogs have shown that the post-op survival rate was much higher than that with MHC-unmatched dogs, indicating that these non-MHC factors are controllable by the administration of immunosuppressants [2,8]. Similarly, as organ transplantation across racial groups have been overcome in humans, genetic differences due to race disparity between donors and recipients is not considered a major factor—in contrast to MHC matching [28,29].

Recently, ABO-incompatible organ transplantation has been widely used in human transplants by desensitization using plasmapheresis, immunoglobulins, B cell depletion via CD-20 antibodies and inhibition of complement activation [30,31]. Although rejection may be induced by different blood type antigens, these desensitization techniques have rarely been used in dogs. Moreover, human antibodies have proven ineffective in dogs [32,33]. Fortunately, most dogs have the same universal blood type, which is DEA1.1 positive [34]; therefore, obtaining canine donors and recipients with matching blood types is not a major constraint, as in human transplantation.

STR genotyping is a useful method for pre-operative selection for transplantation, not only in humans but also in dogs [8,35]. Similarly, in humans, the degree of STR disparities between donor and recipient are associated with postoperative survival time, and moreover, severity of graft-versus-host disease (GvHD) [36]. Therefore, knowledge of the degree of MHC matching is essential for successful results in organ transplantation.

Using the same breed of dog as the donor has been commonly considered for allogenic transplantation to reduce post-op organ rejection. In the present study, we compared the degree of MHC matching between Maltese, purebred, and mongrel dogs. The results showed that percentage of suitable pairs, which are identical or haplo-identical matching, were 4.76% in Maltese-Maltese, 5.13% in mongrel-mongrel, and 6.67% in Maltese-mongrel with no significant differences. The rate of selection of suitable individuals for allogenic transplantation among the possible canine donors would not be very different between the same and different breeds. These results suggest that dogs of the same breed are not necessary for acquiring matching organ donors.

In human, the probability of two randomly selected unrelated individuals are of matching type is very low and varies from race to race. According to a previous report, the probability of HLA matching are 1/11,000 for white American–white American, 1/98,000 for African American–African American, 1/113,000 for African American–white American, 1/29,000 for Asian American–Asian American, and 1/223,000 for Asian American–White American, respectively [37]. In order to increases the probability, worldwide database through the organ transplantation center and marrow donor programs have been used. As results, now, approximately 75–90% of white American and 16–60% of African American patients have the possibilities to find HLA matched donor from unrelated individuals [37,38]. However, even if HLA matched pair using cellular assays of compatibility, only 9.4% of donor-recipient pair were matched for all alleles of DRB1, DQ and DP in MHC class II using DNA-based identification [39]. Therefore, methods are being developed to successfully transplant from HLA unmatched and unrelated donor, as mentioned earlier. In this study, MHC matching probability was relatively high between unrelated 2 dogs compared to previous human reports. This may have been due to the possibilities that dog have relatively less DLA disparity or we used different experimental approaches using microsatellite markers as compared previous studies using serological or DNA-
based methods. In the future, active organ transplantation would be possible if organ donation program and cell bank systems of the companion animals are established.

There were a few limitations in the present study. First, only one breed, Maltese, was included in this experiment as the purebred group. Hence, additional purebred groups might be added for future studies. Furthermore, collecting samples from dogs from various regions and countries will be required to improve the reliability of the experimental results.

Despite MHC-matched transplantation, many animal and human patients have suffered from chronic rejection and diverse complications of immunosuppressants. Infection, malignances, nephrotoxicity, hypertension, gingival overgrowth, diabetes mellitus have been reported as complications of post-transplant immunosuppression, which deteriorates the quality of life for recipients [2,5]. In order to improve the quality of life, development of new immunosuppressants or techniques with fewer side effects would be necessary. Recently, studies on organ transplantation without immune rejection using transplant tolerance created through mixed chimerism and patients-specific artificial organs developed using autologous stem cells have been reported [8,40]. These might lead to optimistic improvement in rejection-free organ transplantation without the use of immunosuppressants in the future.

In conclusion, the present study is the first to compare the degree of DLA matching between purebred Maltese and mongrel dogs. Any breed of canine can be considered as organ donors. Our findings would be beneficial not only for veterinary clinical field but also for medical research using canine models.

**SUPPLEMENTARY MATERIALS**

**Supplementary Fig. 1**
Polyacrylamide gel electrophoresis of amplified MHC class I microsatellite marker. All of the dogs have 1 or 2 bands which were shown in Maltese (A) and mongrel (B) dogs. The location of their bands was equal or different from each other. Each band was labeled by alphabet latter according to size. All bands were located between 500 and 600 bp.

**Supplementary Fig. 2**
Polyacrylamide gel electrophoresis of amplified MHC class II microsatellite marker. All of the dogs have 1 or 2 bands which were shown in Maltese (A) and mongrel (B) dogs. The location of their bands was equal or different from each other. Each band was labeled by alphabet latter according to size. All bands were located between 400 and 500 bp.

**REFERENCES**

1. Gregory CR, Kyles AE, Bernsteen L, Mehl M. Results of clinical renal transplantation in 15 dogs using triple drug immunosuppressive therapy. Vet Surg 2006;35:105-112.
2. Hopper K, Mehl ML, Kass PH, Kyles A, Gregory CR. Outcome after renal transplantation in 26 dogs. Vet Surg 2012;41:316-327.

3. Phillips H, Aronson LR. Use of end-to-side arterial and venous anastomosis techniques for renal transplantation in two dogs. J Am Vet Med Assoc 2012;240:298-303.

4. Rezapour S, Yarmohammadi A, Tavakkoli M. One-year survival rate of renal transplant: factors influencing the outcome. Transplant Research and Risk Management 2017;9:49-56.

5. Park KM, Nam HS, Hussein KH, Woo HM. Surgical management of vesicoureteral reflux with recurrent urinary tract infection after renal transplantation in a dog. J Am Vet Med Assoc 2016;248:309-314.

6. Park KM, Nam HS, Woo HM. Successful management of multidrug-resistant Pseudomonas aeruginosa pneumonia after kidney transplantation in a dog. J Vet Med Sci 2017;75:1529-1533.

7. Niemeyer GP, Welch JA, Tillson M, Brawner W, Rynders P, Goodman S, Dufesne M, Dennis J, Lothrop CD Jr. Renal allograft tolerance in DLA-identical and haploidentical dogs after nonmyeloablative conditioning and transient immunosuppression with cyclosporine and mycophenolate mofetil. Transplant Proc 2005;37:4579-4586.

8. Tillson M, Niemeyer GP, Welch JA, Brawner W, Swaim SF, Rynders P, Lenz SD, Dean B, Lothrop CD Jr. Hematopoietic chimerism induces renal and skin allograft tolerance in DLA-identical dogs. Exp Hematol 2006;34:1759-1770.

9. Mathes DW, Noland M, Graves S, Schlenker R, Miwongtum T, Storb R. A preclinical canine model for composite tissue transplantation. J Reconstr Microsurg 2010;26:201-207.

10. Chong AS, Alegre ML, Miller ML, Fairchild RL. Lessons and limits of mouse models. Cold Spring Harb Perspect Med 2013;3:a015495.

11. Sato M, Keshavjee S, Liu M. Translational research: animal models of obliterative bronchiolitis after lung transplantation. Am J Transplant 2009;9:1981-1987.

12. Hong SH, Eun SC. Experimental forelimb allotransplantation in canine model. BioMed Res Int 2016;2016:1495710.

13. Sergi C, Abdualmjind R, Abuetab Y. Canine liver transplantation model and the intermediate filaments of the cytoskeleton of the hepatocytes. J Biomed Biotechnol 2012;2012:131324.

14. Drukker M, Katz G, Urbach A, Schuldiner M, Markel G, Itskovitz-Eldor J, Reubinoff B, Mandelboim O, Benvenisty N. Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci U S A 2002;99:9864-9869.

15. Neefjes J, Jongma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 2011;11:823-836.

16. van Kasteren SI, Overkleeft H, Ovaa H, Neefjes J. Chemical biology of antigen presentation by MHC molecules. Curr Opin Immunol 2014;26:21-31.

17. Debenham SL, Hart EA, Ashurst JL, Howe KL, Quail MA, Ollier WE, Binns MM. Genomic sequence of the class II region of the canine MHC: comparison with the MHC of other mammalian species. Genomics 2005;85:48-59.

18. Wagner JL, Burnett RC, DeRose SA, Francisco LV, Storb R, Ostrander EA. Histocompatibility testing of dog families with highly polymorphic microsatellite markers. Transplantation 1996;62:876-877.

19. Bettens F, Passweg J, Schanz U, Chalandon Y, Heim D, Gungor T, Stussi G, Nicoloso G, Baldomero H, Gratwohl A, Tiercy JM. Impact of HLA-DPBI haptotypes on outcome of 10/10 matched unrelated hematopoietic stem cell donor transplants depends on MHC-linked microsatellite polymorphisms. Biol Blood Marrow Transplant 2012;18:608-616.
20. Bick SL, Bick DP, Wells BE, Roesler MR, Strawn EY, Lau EC. Preimplantation HLA haplotyping using tri-,
tetra-, and pentanucleotide short tandem repeats for HLA matching. J Assist Reprod Genet 2008;25:323-331.
PUBMED | CROSSREF
21. Schiller JJ, Hopp KA, Pietz BC, Bick DP, Lau EC, Ellis TM. A simplified method for screening siblings for
HLA identity using short tandem repeat (STR) polymorphisms. Hum Immunol 2013;74:562-566.
PUBMED | CROSSREF
22. Park KM, Kang HS, Hussein KH, Kim HM, Kwak HH, Woo HM. Identifying the degree of major
histocompatibility complex matching in genetically unrelated dogs with the use of microsatellite markers.
Transplant Proc 2015;47:780-783.
PUBMED | CROSSREF
23. Shyti E, Idrizi A, Sulcebe G. Histocompatibility testing for organ transplantation purposes in Albania: a
single center experience. Balkan Med J 2014;31:121±125.
PUBMED | CROSSREF
24. Morath C, Zeier M, Döhler B, Opelz G, Süsal C. ABO-incompatible kidney transplantation. Front
Immunol 2017;8:234.
PUBMED | CROSSREF
25. Wagner JL, Burnett RC, Storb R. Organization of the canine major histocompatibility complex: current
perspectives. J Hered 1999;90:35-38.
PUBMED | CROSSREF
26. Johnston SA, Tobias KM. Veterinary Surgery: Small Animal. 2nd ed. Elsevier Health Sciences, St. Louis,
MO, 2013.
PUBMED | CROSSREF
27. Kuhr CS, Allen MD, Junghanss C, Zaucha JM, Marsh CL, Yunosov M, Zellme E, Little MT, Torok-
Storb B, Storb R. Tolerance to vascularized kidney grafts in canine mixed hematopoietic chimeras.
Transplantation 2002;73:1487-1492.
PUBMED | CROSSREF
28. Allen JG, Weiss ES, Arnaoutakis GJ, Russell SD, Baumgartner WA, Conte JV, Shah AS. The impact of
race on survival after heart transplantation: an analysis of more than 20,000 patients. Ann Thorac Surg
2010;89:1956-1963.
PUBMED | CROSSREF
29. Pillay P, Van Thiel DH, Gavaler JS, Starzl TE. Effect of race upon organ donation and recipient survival in
liver transplantation. Dig Dis Sci 1990;35:1391-1396.
PUBMED | CROSSREF
30. Becker LE, Süsal C, Morath C. Kidney transplantation across HLA and ABO antibody barriers. Curr Opin
Organ Transplant 2013;18:445-454.
PUBMED | CROSSREF
31. Morath C, Becker LE, Leo A, Beimler J, Klein K, Seckinger J, Khim LP, Schemper P, Macher-Goeppinger
S, Wahrman M, Böhmig GA, Opelz G, Süsal C, Zeier M, Schwitter V. ABO-incompatible kidney
transplantation enabled by non-antigen-specific immunoadsorption. Transplantation 2012;93:827-834.
PUBMED | CROSSREF
32. Impellizzeri JA, Howell K, McKeever KP, Crow SE. The role of rituximab in the treatment of canine
lymphoma: an ex vivo evaluation. Vet J 2006;171:556-558.
PUBMED | CROSSREF
33. Jubala CM, Wojcieszyn JW, Valli VE, Getzy DM, Fosmire SP, Coffey D, Bellgrau D, Modiano JF. CD20
expression in normal canine B cells and in canine non-Hodgkin lymphoma. Vet Pathol 2005;42:468-476.
PUBMED | CROSSREF
34. Medina Valentin AA, Gavazza A, Lubas G. Prevalence of dog erythrocyte antigen 1 in 7,414 dogs in Italy.
Vet Med Int 2017;2017:5914629.
PUBMED | CROSSREF
35. Kuhr CS, Yunosov M, Sale G, Loretz C, Storb R. Long-term tolerance to kidney allografts in a preclinical
canine model. Transplantation 2007;84:545-547.
PUBMED | CROSSREF
36. Li S, Kawata H, Katsuyama Y, Ota M, Morishima Y, Mano S, Kulsiki JK, Naruse T, Inoko H. Association of
polymorphic MHC microsatellites with GVHD, survival, and leukemia relapse in unrelated hematopoietic
stem cell transplant donor/recipient pairs matched at five HLA loci. Tissue Antigens 2004;63:362-368.
PUBMED | CROSSREF
37. Bergstrom TC, Garratt RJ, Sheehan-Connor D. One chance in a million: altruism and the bone marrow
registry. Am Econ Rev 2009;99:1309-1334.
PUBMED | CROSSREF
38. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, Hartzman R, Rizzo JD, Horowitz M, Confer D, Maiers M. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med 2014;371:339-348.

39. Hurley CK, Baxter-Lowe LA, Begovich AB, Fernandez-Vina M, Noreen H, Schmeckpeper B, Awdeh Z, Chopek M, Salazar M, Williams TM, Yunis EJ, Kitajima D, Shipp K, Splett J, Winden T, Kollman C, Johnson D, Ng J, Hartzman RJ, Hegland J. The extent of HLA class II allele level disparity in unrelated bone marrow transplantation: analysis of 1259 National Marrow Donor Program donor-recipient pairs. Bone Marrow Transplant 2000;25:385-393.

40. Oura T, Cosimi AB, Kawai T. Chimerism-based tolerance in organ transplantation: preclinical and clinical studies. Clin Exp Immunol 2017;189:190-196.