DONOR SELECTION FOR RENAL TRANSPLANTATION

A study on mixed lymphocyte reactions and kidney allograft survival in unimmunosuppressed dogs

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CONTENTS

CHAPTER 1

INTRODUCTION

1.1. Kidney transplantation and immunological rejection

The prime cause of failure of a transplanted kidney is immunological rejection of the graft. Graft rejection will not occur, when the transplanted organ is obtained from a donor which is genetically identical to the recipient (*isogenic* transplant). Graft rejection can always occur, when donor and recipient are genetically different (*allogenic* transplant). Unrelated people are always genetically different. Only when donor and recipient are monozygotic twins, complete genetic identity exists. Thus a transplanted organ will be antigenic, when donor and recipient are not monozygotic twins. The immune reaction, evoked by the antigens of such an allograft, consists of two components. In the first place, antibodies will be produced, which are specifically directed against the transplanted antigens (the so called *humoral* immune response). Secondly, immune reactive cells are generated, which specifically can attack the transplanted organ (the *cellular* immune response). The mechanism of the destruction of the allograft is complicated and only partly known. Both different types of antibodies and different types of cells are involved. Microscopically, arteritis and a cellular infiltrate can be seen (Busch et al., 1977). Progressive damaging of the glomeruli and tubuli results in a increasing loss of function of the transplanted kidney.

Two factors determine the strength of the immune reaction of the recipient against the allograft. These factors are the immune response potential of the recipient and the strength of the antigenic stimulus (Lengerová, 1969). The strength of the antigenic stimulus is dependent on the immunogenetic difference between donor and recipient. Consequently, two methods are available to prevent allograft rejection, namely modification of the immune response and selection of compatible donor-recipient pairs.

1.2. Prevention of the allograft rejection

1.2.1. Modification of the immune response

Aspecific suppression of the immune response can be accomplished by the administration of drugs, as corticosteroids and azathiopurine. Some clinicians advocate a short course of anti-lymphocyte globulin (ALG) in addition (e.g. Monaco et al., 1977). Immunosuppressive drugs have potentially dangerous

side-effects. Besides suppression of the immunity against the transplanted foreign tissue, the immunity against pathogenic micro-organisms is lowered as well. Consequently, local infections and infectious diseases are a feared cause of morbidity and mortality for those under immunosuppressive therapy. Another complication of long term treatment with immunosuppressive agents is a higher incidence of malignant tumours in patients with an allograft (Penn, 1977).

Specific suppression of the immune response against the transplanted organ would be a better form of therapy. The immunity against pathogenic micro-organisms would be unaffected. Indeed, specific prolonged acceptance of an allograft can be achieved in mouse and rat models (Jeekel, 1971; Marquet et al., 1971). The prolonged acceptance of allografts in these models is probably caused by *enhancement*. Enhancement of an allograft is defined as the prolonged acceptance of an allograft, due to the presence in the recipient of allo-antibodies directed against the allo-antigens of the donor (Snell, 1970). Enhancing antibodies do not damage but protect the allograft. However, reliable methods to induce enhancement in larger mammals, including man, have not yet been developed sofar.

1.2.2. Histocompatibility matching

Selective suppression of the rejection reaction against tissue antigens on the transplanted organ, without affecting the general immune status of the recipient, is presently not possible in man. Consequently, clinical allotransplantation cannot be performed without the use of immunosuppressive drugs. Only in the case where the so called *transplantation* or *histocompatibility* antigens of the donor organ (which provoke the immune response of the host) differ only slightly from those of the recipient, can low doses of immunosuppressive drugs be used to prevent rejection. Thus, the risk for drug induced diseases should be lower, when the donor is *compatible* for these antigens. Therefore, the selection of compatible donor-recipient combinations *(histocompatibility matching)* is another logical approach to improving the prospects of allograft recipients.

1.3. Major histocompatibility complex

1.3.1. Genetics

A variety of different histocompatibility antigens is present on the cells of each individual. Some of these antigens (the *strong* or *major* histocompatibility antigens) are more likely to elicit a rejection reaction in the host than others (the so called *weak* or *minor* histocompatibility antigens). The genetic code for all these

antigens resides in many genes on different chromosomes. For instance, skin graft survival in mice is controlled by at least 29 different genes (Graff and Bailey, 1973). However, one particular chromosomal region, which codes for important histocompatibility antigens, has been identified in many species. This region is called the *Major Histocompatibility Complex (MHC)*.

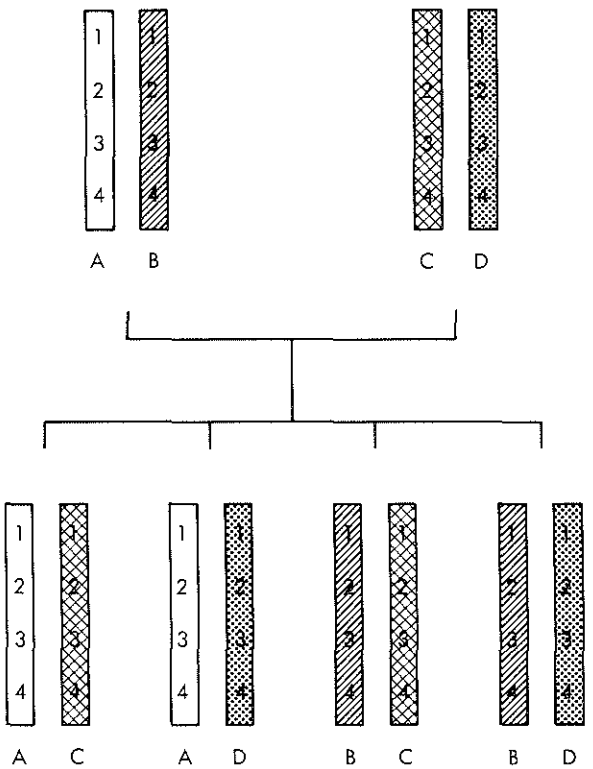


Figure 1.1. Pedigree of a family with different MHC genotypes. Explanation: see text.

Extensive knowledge on the genetics of the MHC has been gathered in many species including man (for a review see D. Götze, ed.: The major histocompatibility system in man and animals, 1977). The MHC contains many, maybe hundreds of loci. At present, the gene products of only some of these loci can be determined.

In families, the genes of the MHC are inherited as genetic entities which segregate en bloc. A simplified scheme of the normal inheritance pattern is presented in figure 1. The rectangles in the figure present the MHC's of both homologous chromosomes of each individual. The genes of the MHC of one chromosome, which segregate together within a family (the rectangle), is called a *haplo-*

type. Thus, each individual has two haplotypes. The haplotypes of the father are here labelled A and B, and those of the mother C and D. The possible "genotypes" of their offspring are AC, AD, BC and BD. The homologous loci on the haplotypes are labelled 1, 2, 3 and 4 in this example. Each locus can be occupied by alternative (mutually exclusive) genes or *alleles*. The number of alleles varies per locus, but within a family never more than four alleles are present for each locus. For simplicity, in the example of figure 1 the alleles of the series 1, 2, 3 and 4 are labelled 1(A), 1(B), 1(C), 1(D); 2(A), 2(B) etc. It is clear that, when two children of the same family have the genotype AC, they will be identical for the genes of series 1, 2, 3 and 4 of both haplotypes. When two children are known to have 1(A), then usually both will have also 2(A), 3(A) and 4(A) in common. Thus, within a family, matching for one or just a few MHC antigens generally results in matching for all MHC antigens of a haplotype. Within a family three MHC matches are possible: two individuals can be two haplotypes different (e.g. AC and BD); they can be one haplotype different (or "haploidentical", e.g. AC and AD), or they can be MHC identical, when they share the determinants of both haplotypes (e.g. AC and AC).

Sometimes this simple "A-B-C-D" pattern of inheritance is disturbed by the occurrence of a so called *crossing-over* between the two homologous chromosomes, which carry the MHC. This happens when, during the meiosis of the spermatocyte or the oöcyte, these chromosomes exchange parts and consequently a new haplotype originates (figure 2). An individual with such a *recombinant* MHC will never be completely MHC identical with its sibs. The frequency of the occurrence of such a recombination between two genetic markers on one chromosome in a given population is a measure for the distance between these genes on the chromosome. Thus, when two loci are located comparatively far from each other on the same chromosome, a recombination between them will occur relatively often. If two loci are close to each other on the chromosome *(closely linked)*, a recombination will be rare. Within the MHC, recombinations are relatively rare. Such recombinations are of interest, as they may be helpful in the mapping of genes within the MHC (e.g. Netzel et al., 1975; Bijnen et al., 1975[a]; 1976). When individuals with such a recombination are used in transplantation experiments, it is sometimes possible to estimate the importance of a particular part of the MHC for graft survival. Thus, for example, by transplantation experiments in recombinant strains of mice the relative importance of the various subregions of the mouse MHC with regard to skin graft survival has become fairly accurately known (David, 1977).

Some loci of the MHC are demonstrated to be extremely *polymorphic*, as for each locus a series of many different alleles exists. Within a family however only four different alleles are present for each locus; identity for one gene

of a haplotype is generally accompanied by identity for the other genes of that haplotype. Thus, completely MHC matched sibling donor-recipient combinations can often be found. The chances to find unrelated compatible donor-recipient combinations will be much more dependent on the polymorphism of the important histocompatibility systems.

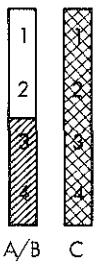


Figure 1.2. Genotype of an individual with a paternal recombination in the MHC. Explanation: see text.

1.3.2. Histocompatibility testing

Two different in vitro techniques for histocompatibility testing will be described here: the lymphocytotoxicity test and the mixed lymphocyte reaction.

1.3.2.1. Testing for SD antigens with the lymphocytotoxicity test

For this test, sera are required which contain specific antibodies directed against histocompatibility antigens. To obtain antisera containing such antibodies two sources are available. In the first place alloantisera can be raised by deliberate immunization of volunteers. Furthermore, during pregnancy sometimes antibodies are produced which are directed against the antigens of the foetus. Thus post-partum sera are another important source of alloantisera (Payne and Rolfs, 1958; van Rood et al., 1958). All sera have to be analyzed for their specificity. Sometimes they contain a mixture of antibodies. Then they have to be purified by absorbing out the undesired antibodies. With a set of properly selected antisera random individuals may be tested.

For this testing generally a complement dependent lymphocytotoxicity method is used, which takes a couple of hours. Peripheral blood lymphocytes of an individual are added to a panel of test sera. If a serum contains cytotoxic antibodies which are directed against antigens of these lymphocytes, then these lymphocytes are killed in the presence of complement. After staining with eosine or trypan blue the viability of the cells can be assessed under the light microscope. Killing of lymphocytes by an antiserum implies that these lympho-

cytes carry the antigen which is recognized by the specific antiserum. The antigens on lymphocytes recognized by this serological technique are called *serologically defined (SD)* antigens, in contrast to the so called *lymphocyte defined (LD)* antigens as described in the next paragraph.

1.3.2.2. Testing for LD antigens with the mixed lymphocyte reaction

Under adequate culture conditions lymphocytes can be stimulated by allogeneic lymphocytes to proliferate and transform into blasts (Bain et al., 1964; Bach and Hirschhorn, 1964). This proliferation and blast transformation is associated with an increase in the amount of DNA in the cultured cell suspension. The increase in DNA can be measured if radioactive labelled thymidine is added to the culture. Generally this is done a number of hours before the end of the culture period which takes several days. The amount of radioactive thymidine which is incorporated into the DNA is then a measure for the growth rate at that time. The reaction of the lymphocytes in such a mixed lymphocyte culture is called the *mixed lymphocyte reaction (MLR).*

Before mixing the cell suspensions at the beginning of a culture, the cells of one of the suspensions can be pretreated with drugs (or x-irradiated) to prevent their growth (Bach and Voynow, 1966). If this is done properly, such cells stay alive and do not lose their antigenic properties. The pretreated cells are called *stimulator* cells. The advantage of such a pretreatment is that only the cells of the non-pretreated suspension, the *responder* cells, can grow. Thus, in such a *unilateral* MLR, all activity measured is the result of an increase in DNA synthesis of the responder cell population. If both cell suspensions are not pretreated, the test is called a *bilateral* MLR. The interpretation of a bilateral mixed lymphocyte culture is obviously more difficult, as the contribution of each separate cell suspension to the measured activity cannot be determined.

Measurable reactivity in the MLR means that two individuals differ for a special kind of histocompatibility antigens, the *MLR* antigens. As these antigens are recognized by lymphocyte culturing techniques, they are also called *lymphocyte defined (LD)* antigens.

1.3.3. Matching for gene products of the MHC

1.3.3.1. SD antigens

After the discovery that anti-leucocyte antibodies sometimes are produced during pregnancy (Payne and Rolfs, 1958; van Rood et al., 1958), large amounts of reliable alloantisera became available for SD testing. Typing for leucocyte antigens became only feasible after the introduction of the computer assisted analysis of the reaction patterns of antisera with large panels of leucocytes (van Rood, 1962; van Rood and van Leeuwen, 1963).

In man presently three allelic series of SD antigens are recognized with large batteries of well-defined antisera (Thorsby, 1974). They are labelled HLA-A, HLA-B and HLA-C. Because of the large number of alleles in each series, theoretically millions of phenotypes would be possible if the various antigens of the different allelic series would be distributed independently of each other in the population. Consequently, matching for the antigens of the MHC would be practically impossible. However matching is possible as some phenotypes are more frequent than others. There are two reasons why some phenotypes are more frequent than others. In the first place, the gene frequencies of some antigens are higher than the gene frequencies of other antigens. Combinations of frequent occurring genes will thus also be found more often than other combinations. Secondly, some combinations of antigens of different allelic series occur more often than expected on the basis of their individual gene frequencies. This non random association of certain alleles of different series is called *linkage disequilibrium*. Linkage disequilibrium between genes of different allelic series is well documented in several outbred species, including man, rhesus monkey and dog. Recent information on the gene frequencies and linkage disequilibria within these species is reviewed in D. Götze, ed.: The major histocompatibility system in man and animals (1977). In fact the occurrence of linkage disequilibrium implicates that some "haplotypes" (and phenotypes) are rare, while others occur more frequent. Therefore, donor-recipient matching may well be practicable for individuals with frequent occurring HLA phenotypes.

1.3.3.2. LD antigens

The MHC does not only contain the genes which code for the SD antigens, but also contains the genetic information which determines the outcome of the MLR. SD and LD antigens are proven to be determined by separate loci in several species. In man one *major* MLR locus is recognized (Yunis and Amos, 1971). Besides this major MLR locus, the MHC probably contains *minor* MLR loci as well (Eijsvoogel et al., 1972; Mempel et al., 1973; Suciu - Foca and Dausset, 1975; Netzel et al., 1975; Bijnen et al., 1977).

In the mouse the gene products of even 5 loci within the MHC (Meo et al., 1973) and one locus outside the MHC, the so called M locus (Festenstein et al., 1972) are involved in the determination of the outcome of the MLR. Also in other species, as the rhesus monkey (Balner et al., 1973), the pig (Vaiman et al., 1973), the dog (van den Tweel et al., 1974) and the rat (Williams et al., 1977), the MLR is controlled by a distinct locus.

To match for MLR or LD antigens, pairs of individuals can be tested together in a mixed lymphocyte culture. The MLR is a direct cross reaction, which measures the presence or absence of an incompatibility between individuals without

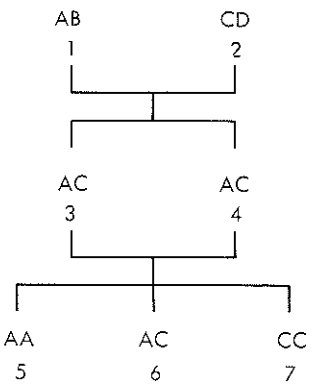
typing them. Twenty-five percent of the siblings are compatible for the LD antigens. The polymorphism of LD antigens however makes a chance compatibility between random unrelated individuals an extreme rarity. Thus large groups of individuals should be cross tested when MLR compatible individuals are looked for.

Theoretically a helpful method for the selection of unrelated MLR negative combinations is *LD typing*, i.e. assigning LD specificities to individuals. LD typing can be done by means of LD typing cells. Such cells are homozygous for LD determinants. They can be obtained from inbred individuals (Jørgensen et al., 1973; Keuning et al., 1975[a], Keuning, 1978). Figure 3 illustrates, by means of an example of a brother-sister mating, how such typing cells can be selected. The father ("1") and the mother ("2") are both heterozygous for the LD antigens and have the genotypes AB and CD respectively. In this example, they have two children ("3" and "4"), both with the genotype AC. The offspring of a brother-sister mating of these individuals have the genotypes AA, AC and CC (individuals "5", "6" and "7" respectively). Individuals "5" and "7" are LD homozygotes. If all individuals of this family are cross tested with each other, an MLR pattern will be seen as illustrated in the figure. For simplicity in this example only a distinction is made between negative and positive reactions. The homozygous individuals "5" (genotype AA) and "7" (CC) can produce a so called *one way reaction*. For instance "5" (AA) does not stimulate other individuals which carry the LD type "A", such as "1" (AB) and "3" (AC). These individuals namely recognize the A determinant as "self". In contrast, "5" (AA) is stimulated by "1" (AB) and "3" (AC) because they carry LD determinants (B and C respectively), which are foreign to "5". With the homozygous cells "5" (AA) and "7" (CC) it is possible to type the other members of the family as well. For instance, it can be seen from the table that individual "6" should be typed "AC", as he exhibits a one way positive reaction with "5" (AA) and "7" (CC).

When several *homozygous typing cells* are available, population studies can be performed. Specificity numbers can be assigned to different LD typing cells, and unrelated individuals can be typed. In practice, not only clearly negative and positive reactions occur. Sometimes a homozygous typing cell causes a weakly positive response. Such a weak *typing response* may also indicate that the responder carries the LD determinant of the typing cell. Unfortunately, LD typing of unrelated individuals is hampered by the fact that unrelated individuals can still be MLR positive, even if they carry the same LD determinants (van Rood, 1978; Keuning, 1978).

In summary, LD matching (i.e. selection of LD or MLR compatible individuals) can be performed by means of a direct MLR or by means of LD typing. Complete LD or MLR identity can only be ascertained with the direct MLR. Therefore, in this thesis, with *LD or MLR identity* is meant that a direct MLR

was performed and that the test outcome was completely negative. *LD or MLR compatibility* means that a direct MLR was performed, and that the test outcome was negative or lowly positive. If LD or MLR compatibility is only ascertained by means of LD typing, than this is specifically indicated in the text.



responder cells	Stimulator cells 1x	2x	3x	4x	5x	6x	7x
1	-	+	+	+	-	+	+
2	+	-	+	+	+	+	-
3	+	+	-	-	-	-	-
4	+	+	-	-	-	-	-
5	+	+	+	+	-	+	+
6	+	+	-	-	-	-	-
7	+	+	+	+	+	+	-

Figure 1.3. MLR between heterozygous and homozygous family members. Explanation: see text.

1.4. SD compatibility and human renal allograft survival

Histocompatibility matching may contribute to improved kidney allograft survival rates, improved patient survival rates and a reduced morbidity in renal allograft recipients (§ 1.2.2.). Patient morbidity is difficult to quantitate and little knowledge is available on the correlation between morbidity and histocompatibility match grade. Kidney allograft survival data are presumably a more sensitive index for the evaluation of the effect of histocompatibility matching than patient survival data, as many patients, who reject the transplanted kidney, return to dialysis and stay alive. Therefore, kidney allograft survival data are described most extensively in the literature; these data will be discussed below.

1.4.1. Related donors

Kidney allografts from MHC identical sibling donors have an excellent prognosis. After one year about 85% - 90% of the kidneys are still functioning. This is significantly better than the one year survival of grafts from parental donors and one

haplotype different sibling donors, which is about 70% (65% - 85%) (Singal et al., 1969; Brynger et al., 1977; Opelz et al., 1977[e]; Solheim et al., 1977). The one year graft survival of kidneys obtained from two haplotype different sibling donors is still worse, namely ± 50% (Opelz et al., 1977[e]).

1.4.2. Unrelated donors

Related individuals are an important source of donor organs, but more than twice as much of the registered kidney allografts are presently obtained from cadaver donors (Human Renal Transplant Registry, 1977). On the whole, the one year survival of cadaveric kidney allografts however is only ± 50% after one year. Attempts to improve renal allograft survival by means of matching donor and recipient for HLA antigens have been less effective in unrelated than in related donor-recipient combinations.

The literature on the precise effect of HLA matching on cadaveric allograft prognosis seems contradictory and is at least confusing. In Europe, a significant correlation between match grade and graft prognosis is observed in several centers (van Hooff et al., 1972; Dausset et al., 1974; Scandiatransplant report, 1975; Festenstein et al., 1976; Sachs et al., 1977; Solheim et al., 1977). In these centers a one year graft survival of 55% - 70% is found in the *full-house matched* (i.e. matched for two HLA-A and two HLA-B antigens) groups compared to 40%-57% in the mismatched groups. No significant correlation was initially found by some American groups (Belzer et al., 1974; Opelz et al., 1974[a]). More recently a slight but significant influence of HLA matching on functional graft prognosis was also reported by Opelz et al. (1977[a]). Curiously such a favourable effect of matching was only seen in centers with poor overall results.

Some others reported that kidney allograft survival is worse in recipients with circulating preformed antileucocyte antibodies (Terasaki et al., 1971; van Hooff et al., 1972; Opelz et al., 1972; 1974[a] and 1977[a]), but others did not find a difference (Dausset et al., 1974; Ferguson et al., 1977). HLA matching was found to be more effective in patients with such circulating cytotoxic antibodies (van Hooff et al., 1972; Dausset et al., 1974). However this observation is at variance with a recent report of Opelz et al. (1977[a]), who did not find a difference in effect of HLA matching in antibody negative compared to antibody positive recipients.

Matching for HLA-B antigens rather than for HLA-A antigens seems to improve renal allograft survival (van Hooff et al., 1972; Oliver et al., 1972; Dausset et al., 1974; Sachs et al., 1977), suggesting a greater antigenicity of gene products of the HLA-B locus than of the HLA-A locus. Confusingly, others did not record a difference in antigenicity between HLA-A and -B antigens (Opelz et al., 1974[a]; 1977[a]; Scandiatransplant report, 1975).

Finally, van Hooff et al. (1974) and Thompson et al. (1977) found that

matching for certain combinations of antigens, which are known to be in strong linkage disequilibrium with each other (so called *haplotype-matching)*, would improve cadaveric graft survival rates. They suggested, that because of the high linkage disequilibrium of these "haplotypes" with certain LD determinants matching for these SD antigens would imply matching for LD antigens as well. However, the observed linkage disequilibria are insufficient to explain these results (van Rood et al., 1975). Furthermore, other similar studies did not confirm these data (Scandiatransplant Report, 1975; Opelz et al., 1976).

The cause for the differences in results is unclear. Probably, a different composition of the populations under study is in part responsible. However, one general conclusion can be drawn, i.e. that common sensus has been reached on the statistical significance of HLA matching in human kidney graft survival. Its practical significance for the various subsets of patients remains a matter for further evaluation.

1.4.3. Discrepancy in the results of SD matching in related and unrelated donor-recipient pairs

HLA identical related grafts have an excellent prognosis of 85% - 90% after one year (§ 1.4.1.), while full-house HLA identical cadaveric grafts have a poorer prognosis of 55% - 70% after one year (§ 1.4.2.).

One possible cause for the discrepant survival rates of matched related and unrelated kidney allografts is that matching for cadaveric grafts is only performed for the HLA-A and -B antigens. In § 1.3.1. it is explained that matching for the defined antigens of the MHC in related donor-recipient combinations implies that a good match is obtained for all other undefined gene products of the MHC. In unrelated donor-recipient pairs this is not necessarily the case. Thus it is possible that the MHC contains one locus, different from but in linkage disequilibrium with the loci typed for, which is very important for graft survival. If this is the case, it could explain the differences in survival rates. It would mean furthermore that all efforts should be directed to develop a typing method for the gene products of this *graft prognosis locus* (van Rood et al., 1975).

The H-2 complex of mice carries at least three different genes which are relevant for skin allograft rejection (Klein, 1975). Thus, a second possible explanation for the discrepancy of the results is that the defined genes each have a histocompatibility effect of their own and that some extra loci still have to be identified. Thus, Opelz et al. (1977[b]) calculated that each mismatch for an HLA antigen causes a 4% decrease of the one year graft survival.

A third possibility is that the cumulative effect of non-MHC antigens plays an important role. Non-MHC differences are more frequent in unrelated than in related donor-recipient pairs. This is another possible explanation for the lower

survival rates of cadaver donor kidneys. Opelz et al. (1977e) calculated that differences for these non-MHC antigens are responsible for a rejection rate of 15% of the grafts in one year in related donor-recipient combinations. In unrelated donor-recipient combinations this figure may even have to be multiplied by two.

Finally, multiple genes may be involved in the genetic control of kidney allograft survival, some of them concentrated on one chromosomal region (the MHC), others located on different chromosomes.

1.5. LD compatibility and human renal allograft survival

1.5.1. Related donors

The incubation time of an MLR is several days. Therefore, a proper prospective selection of MLR compatible donor-recipient combinations is impossible in cadaver kidney transplantation. Such a prospective study can only be performed with living donors. Living donors are mostly related donors. Related donor-recipient pairs can be classified as MHC identical, one haplotype different or two haplotypes different. Because of the strong linkage between the MLR genes and the other genes of the MHC, a reliable estimate of the value of the MLR for donor selection can only be made if only one (or two) haplotype different donor-recipient pairs are considered. Thus some publications which suggest a positive correlation between stimulation in MLR and kidney allograft rejection are somewhat difficult to evaluate as the haplotype categories are not clearly taken into account in the analysis (Jeannet 1969/1970; Hamburger et al., 1971; Segall et al., 1975; Uehling et al., 1977). However, a positive correlation is also found when all analyzed donor-recipient pairs are explicitly different for one HLA haplotype (Ringden et al., 1976; Solheim et al., 1977).

1.5.2. Unrelated donors

In the unrelated donor-recipient combinations, i.e. in cadaver kidney transplantation, the situation is somewhat different, as identity for MLR antigens does not imply identity for other gene products of the MHC. A positive correlation between MLR and graft rejection is observed in some retrospective clinical studies (Cochrum et al., 1973; Festenstein et al., 1976; Opelz and Terasaki, 1977d). However, Sachs et al. (1977) found no statistical significant difference of kidney graft survival in MLR compatible and MLR incompatible HLA-A and -B matched unrelated donor-recipient combinations.

The number of reports on the value of LD matching for donor selection is much smaller than the number of reports on the relevance of SD matching for

donor selection. However most reports demonstrate that LD compatible grafts have a better prognosis than LD incompatible grafts. Thus it seems justifiable to pursue the attempts which may enable a prospective identification of LD compatible donor-recipient combinations for cadaver kidney transplantation.

1.6. LD compatibility and experimental allograft survival

Research in experimental models can provide additive information on the value of tissue typing methods for donor selection. Many studies have been performed in various species to assess the role of LD compatibility for different organ allografts. The results are variable and depend on the experimental model. For instance no correlation is found between allograft survival and reactivity in mixed lymphocyte cultures for experimental skin graft survival in rhesus monkeys (Balner and van Vreeswijk, 1975), for kidney graft survival in rats (Fabre and Morris, 1974) and graft versus host and host versus graft reactions after bone marrow transplantation in dogs (Vriesendorp et al., 1976). On the other hand many publications show a favourable influence of MLR matching on allograft survival, for instance for skin graft survival in unrelated human volunteers (Koch et al., 1973) and in related human volunteers (Sasportes et al., 1972). Such a favourable effect is also found for kidney graft survival in dogs (M.L. Bach et al., 1975; Westbroek et al., 1975) and rhesus monkeys (van Es et al., 1977). In mice, H-2 LD disparity is always associated with graft versus host reactions and often (but not always) associated with skin graft rejection (e.g. Bach and van Rood, 1976). However, the presence of an M locus disparity does not influence skin and bone marrow graft survival in MHC identical mice, although the MLR in these donor-recipient combinations is positive (Festenstein et al., 1973).

The cause for the discrepancy between the reports which deny and which support the importance of matching for MLR to allograft survival is unknown. The sensitivity of the used techniques may play a role, as well as species specific and organ specific differences. It is clear that more work has to be done to obtain a better insight into the role of MLR matching for allograft survival.

1.7. The dog as an experimental model for histocompatibility and transplantation research

Throughout the years the dog has been a favourable model for transplantation experiments, because of its size, ready availability and good temper, which facilitates easy handling. In the last decade many investigators started histocompatibility studies in this species because of the growing demands of an immunogen-

etically defined preclinical experimental outbred animal model. The short generation time and the large litter sizes of dogs facilitates the breeding of required phenotypes for specific immunogenetic purposes and genetic studies. Developments in this field made international co-operation a necessity. International workshops on canine immunogenetics have been organized in 1972 in Rotterdam, the Netherlands, and in 1975 in Portland, Oregon, U.S.A. . The "Joint Reports" (1973, 1976) present the accumulated knowledge of the canine MHC and other genetic markers in the dog as far as known at that time.

An extensive review of the present knowledge of the canine MHC is recently given by Vriesendorp et al. (1977[b]). It appears that the canine MHC closely resembles the MHC in man. Three series of serologically detectable antigens are defined, DLA-A, DLA-B and DLA-C with 7, 5 and 3 defined alleles respectively. More alleles may be defined in the future, as all series still have "blanks". A high linkage disequilibrium exists between the alleles of the three series. The cause for this linkage disequilibrium may be a so called "bottleneck" or "founder" effect (Vriesendorp, 1973). According to that author, periods and/or areas may have existed in dog history, in which only a limited number of animals was available for reproduction. The DLA haplotype of these ancestors will be overrepresented in the present dog populations. Another consequence of this "founder effect" is the limited polymorphism for example of red and white cell enzyme markers in this species (Meera Khan et al., 1973), indicating that the dog is less heterogeneous than man.

The first reports on mixed lymphocyte reactions in dogs appeared in 1968 (Serre and Clot), 1969 (Rudolph et al.) and 1971 (Templeton and Thomas). Many different techniques have been used since (Gluckman, 1973; Grosse-Wilde et al., 1973; van den Tweel et al., 1974; M.L. Bach et al., 1975; Goldman and Flad, 1975). The outcome of the MLR in dogs appeared to be determined by the MHC analogous to the situation in other species. A recombination between the SD and MLR loci was reported which proved that the MLR is governed by a locus distinct from but closely situated to the SD loci (van den Tweel et al., 1974). This locus has been labelled DLA-D (Joint Report of the Second International Workshop on Canine Immunogenetics, 1976). A strong linkage disequilibrium between SD and MLR loci was observed (van den Tweel et al. 1974; Grosse-Wilde et al., 1975[b]). Furthermore LD typing with homozygous reference cells appeared to be feasible in dogs. With the use of these typing cells a dog family was found in which two "LD types" segregated on one haplotype (Grosse-Wilde et al., 1975[b]). This suggested that the MLR in dogs is governed by more than one LD locus.

Transplantation experiments with various organs clearly demonstrated the relevance of matching for antigens of the canine MHC for graft prognosis (figure 4). The differences in mean survival times for the different transplanted organs

are caused, among others, by organ specific differences (Vriesendorp et al., 1977b). The resemblance between the canine MHC and the human MHC justifies the use of the dog as a model for further transplantation research (Vriesendorp et al., 1977a). The dog model is an excellent preclinical model not only for its immunogenetic characterizations, but also because outbred dogs are widely available. Even in a closed beagle colony outbreeding can be effectuated, if the ancestor composition is sufficiently large (Rehfeld, 1970).

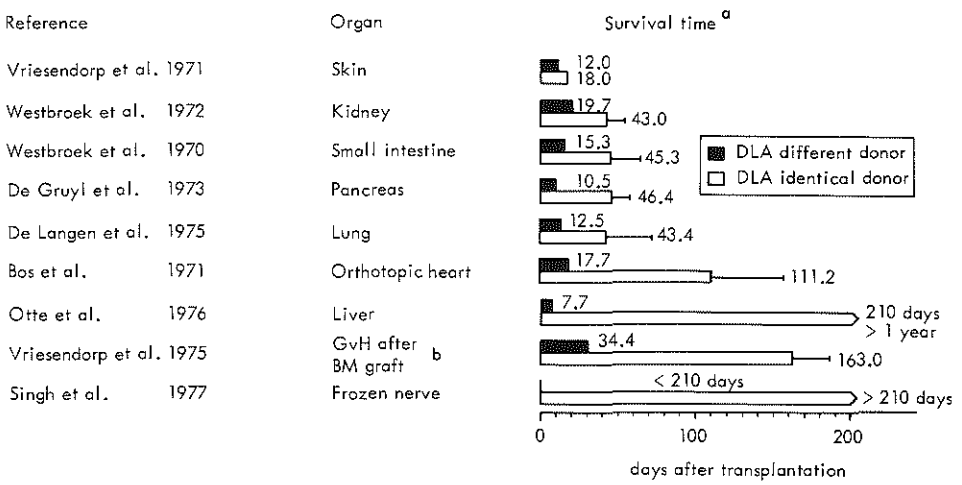


Figure 1.4. Effect of DLA matching on allograft survival in littermate donor-recipient pairs.
a. One standard deviation of the mean is given. Each mean is computed from the survival times of at least 6 different animals, except for liver grafts, because only 2 animals have been grafted per group sofar.
b. Recipients received 750 rads TBI and 100 mg silica/kg bodyweight i.v..
Reproduced, with permission, from:
Genetics and transplantation immunology of the DLA complex.
Vriesendorp, H.M., Bijnen, A.B., Westbroek, D.L. and van Bekkum, D.W. (1977) Transpl. Proc. 9, 293-296, Grune & Stratton Inc., New York.

1.8. Objectives

The goals of the transplantation immunological research in our laboratory are:
a) to create and maintain a preclinical model which is optimally suited for transplantation research, namely the dog,
b) to assess the importance of histocompatibility matching for allograft survival in related and unrelated donor-recipient combinations, and
c) to test various forms of treatment of recipients which may prevent or delay graft rejection in this model.

As a part of these objectives the studies presented in this thesis were initiated. At the time of the start of these studies the relevance of the MLR to graft survival in unrelated donor-recipient pairs was a main topic in histocompatibility research. For an optimal interpretation of the test results for genetic studies and organ transplantation experiments it was felt essential to acquire an extensive insight into the kinetics of the MLR in various defined genetically different cell mixtures. These results will be described in chapter 3.

For the selection of defined donor-recipient combinations it was thought to be useful to analyse the genetics of the MLR's which were primarily performed for transplantation experiments. This analysis is discussed in chapter 4. Furthermore it seemed essential to determine the importance of matching for MHC versus non-MHC gene products in renal allograft survival. Therefore, pairs of LD and/or SD identical beagles were selected. These animals were assumed to be identical for the other genes of the MHC as well. Kidneys were exchanged between these pairs and graft rejection was considered to be caused by differences for non-MHC antigens. As the number of non-MHC incompatibilities in these dogs had to be larger than in littermate donor-recipient combinations, the differences in survival times between the DLA identical non-sibling and littermate donor-recipient combinations should be indicative of the importance of matching for the MHC versus non-MHC antigens. These experiments are described in chapter 5.

In chapter 6 the relevance of the subregions of the MHC for allografting is investigated. Therefore a number of kidneys was transplanted in donor-recipient combinations of which donor or recipient was an MHC recombinant. Furthermore, renal allografts were performed in unrelated donor-recipient combinations which were typed for the known SD antigens of the MHC and tested in MLR. The latter experiment was performed to evaluate the relative importance of matching for MLR compared to matching for SD antigens for kidney graft survival. It was hoped that insight could be gained into the importance of the subregions of the MHC. These analyses are described in chapter 6.

The order of the chapters does not reflect the chronological order of the experiments. Some kinetical experiments were performed when an MLR result deviated from the expected pattern. The genetic studies were performed partly during donor-recipient selection programmes and the described recombinations were accidently discovered. Grafting of the recombinants was performed when they became available after more elaborate genetic studies. It was the purpose of the experiments described in this thesis to collect theoretical and practical information on the efficacy of histocompatibility matching for renal allografting in a well defined experimental animal, namely the tissue typed dog.

CHAPTER 2

DESCRIPTION OF TECHNIQUES

2.1. Serology

For the experiments, described in this thesis, the one stage microlymphocytotoxicity test as described by Kissmeyer-Nielsen and Kierbye (1967) and adapted for canine lymphocytes by Vriesendorp (1973) was used. In this test, antiserum and complement are both added to the cells directly at the beginning of the incubation period of 30 minutes at 37° C. Production and evaluation of the used test sera are described by Vriesendorp et al. (1971). With a battery of 70-90 of these tests sera the gene products of three linked loci can be recognized (Vriesendorp, 1977b). Nomenclature decisions on the officially recognized specificities have been published in the Joint Report of the Second International Workshop on Canine Immunogenetics (1976).

2.2. Mixed lymphocyte reaction

2.2.1. Blood sampling

Blood is drawn under sterile conditions by venipuncture from the forelegs or the external jugular vein and collected in a sterile siliconized glass bottle. Heparin (1 ml of Thromboliquine (Organon, Oss, Holland) per 100 ml of blood) is used as anticoagulant. Defibrinated in stead of heparinized blood is sometimes used, when the cell suspensions are prepared on the day of blood sampling, to prevent clumping of cells. Defibrination is not done routinely, as inadvertent clotting may occur, extra manpower is needed to shake the bottles and the cell yield is considerably smaller compared to the cell yield with heparinized blood.

2.2.2. Short term preservation of blood

For logistical reasons blood sometimes is stored for one or two days at room temperature after dilution with at least an equal amount of culture medium (see § 2.2.5).
Special care is taken that little or no air is left in the bottles and that they are closed air-tight. With this storage method cell viability remains over 90% (assessed by the trypan-blue dye exclusion test) and no loss of reactivity in the mixed lymphocyte culture is noted.

2.2.3. Preparation of lymphocyte suspensions

Handling of materials, media and blood components is performed, when possible, in a filtered air unit (obtained from REPGO-TNO, Rijswijk, the Netherlands).

For the preparation of lymphocyte suspensions the following procedure is pursued: The blood/medium mixture is centrifuged in aliquots of 30 ml in siliconized 40 ml glass tubes for 10 minutes at 900 g (1800 rotations per minute (= rpm) with the Heraeus UJ III E centrifuge). Then the plasma and buffycoat are collected and centrifuged during 10 minutes at 400 g (800 rpm), also in 40 ml glass tubes. The supernatant (with the bulk of the thrombocytes) is removed down to 0.5 cm above the sediment and discarded. The cells are resuspended in medium RPMI-1640 (Flow, cat. no. 2-063M), underlayered with Ficoll-Isopaque (density 1.077 g/ml) and centrifuged for 25 minutes at 900 g (1800 rpm) without breaking. The lymphocyte-rich interface is collected, quickly divided into two plastic Falcon tubes with 8 ml of RPMI-1640 and centrifuged for 10 minutes at 700 g (1400 rpm). This washing procedure is repeated twice. After the last turn the supernatant is removed completely. The cells are resuspended in 1 ml of culture medium for each 10 ml of blood sampled, counted in a haemocytometer (after dying with Türk) and readjusted to a final concentration of 7.5 x 10^6 lymphocytes per ml. The yield is generally 1-2 x 10^6 lymphocytes per ml of blood. The percentage of contaminating granulocytes is generally about 10%.

2.2.4. Pretreatment of stimulator cells

Irradiation from 1000 to 5000 rad completely prevents mitosis without inhibiting the stimulating capacity of the cells (own observations). Therefore, a dose of 2500 rad (approximately 120 rad per minute, 250 KV) is chosen to abolish the mitotic potential of the stimulator cells. Pretreatment with mitomycin - C (A. Christiaens S.A., Brussels, Belgium) at 25 μ g/ml for 30 minutes is equally effective for preparing stimulator cells for dog lymphocyte cultures (van den Tweel et al., 1974). This method is more cumbersome and only used in our laboratory when irradation cannot be performed.

2.2.5. Culturing

Culturing is performed in a technique similar to the technique of Grosse-Wilde (1973). As a rule, each combination of cells is tested in quadruplicate. As a culture medium RPMI-1640 supplemented per 100 ml with 10 mMole freshly thawed L-Glutamine (Gibco cat. no. 503), 1 ml of a solution containing 10,000 I.U. penicillin and 10,000 μ g streptomycin per ml (Flow, cat.no. 7-010 C) and 10

ml heat inactivated normal dog pool serum is used. The serum pool is prepared from 6 or more unimmunized sires and freshly frozen at - 70° C. Each serum-pool is always tested in different concentrations in a control MLR and not kept for longer than one year.

First, 0.2 ml of the culture medium is pipetted into the wells of a flat bottomed micro-titer plate (Falcon, cat. no. M 3040). Then 0.02 ml (containing 1.5 x 10^5 cells) of the responder and stimulator cell suspensions each are added to each well with a tuberculin syringe and a specially adapted Hamilton dispenser (Hamilton Inc., cat. no. PB-600). The plates are covered with a sterile lid (Falcon, cat. no. M 3041) and incubated at 38° C in a slightly inclined position (30°) in a water-saturated atmosphere containing 5% CO_2.

2.2.6. Labelling

Sixteen hours before harvesting radioactive labelled thymidine is added. Until march 1975, 2 μ Ci of methyl-3H-thymidine (specific activity 5 Ci/mMole, Amersham, cat. no. TRA 120) was pipetted into each well; currently 0.8 μCi of methyl-3H-thymidine with a specific activity of 2 Ci/mMole (Amersham, cat. TRA 310) is used.

2.2.7. Harvesting

Unless stated otherwise, harvesting is performed on the sixth day of culture. This is done by precipitating the cells from the cultures on glass fibre filters (Titertek, cat. no. 77-300-06) by means of a multiple sampling harvester (Skatron, Lierbyen, Norway).

2.2.8. Counting

The filters are dried and put in their correct order in disposable glass flasks with a plastic screwcap (Amphabel, cat. no. 430) and 2.5 ml of scintillation fluid (2.5 liter Toluol, Merck, cat. no. 8325 supplemented with 0.025 g POPOP; Packard, cat. no. 6002030 and 15 g PPO, Merck, cat. no. 2946) is added. This amount is sufficient as the cells stay on the filter on the bottom of the vial. Scintillation counting is performed in a Packard Tricarb liquid scintillation spectrophotometer (Model 3375) for one minute periods. The results are expressed as cpm (counts per minute) and recorded on a punch tape for computerized calculation of the means of the quadruplicates and the standard deviations.

2.2.9. Controls

For a correct interpretation of the results appropriate negative and positive con-

trols are essential. As negative control cultures a double dose of responder cells without stimulator cells, and a single dose with autologous stimulator cells are tested; the stimulator cells are tested in combination with allogeneic MHC different stimulator cells. As positive controls all responder cells are cocultured with MHC different stimulator cells and vice versa.

2.3. Renal allografting

2.3.1. Donor nephrectomy

The abdomen is opened through a midline incision. In the absence of anatomic abnormalities the left kidney is used. Artery and vein of the donor kidney are dissected free close to the aorta and vena cava and the ureter is transected close to the bladder. Immediately after removal most kidneys were perfused with 50-100 ml cold (4°C) Isodex, Organon, Oss (supplemented with 5.10^6 I.U. penicillin and 20 ml lidocaine 2% per 500 ml), followed by a simultaneous perfusion of invert sugar 10% and $NaHCO_3$ 1.4%. In later series the kidneys were perfused with NaCl 0.9% (supplemented with 25 mg heparine, Thromboliquine, Organon, Oss and 50 ml procaine 2% per 500 ml).

2.3.2. Renal implantation

The donor kidney is implanted via a midline incision into the contralateral (usually right) iliac fossa of the recipient. First the renal vein is anastomosed end to side to the common iliac vein while the cold perfusion is continued. Thereafter the renal artery is anastomosed end tot end to the external iliac artery. If the donor kidney has two arteries, a second end to end anastomosis is made with the caudal artery. The ureter is anastomosed to the bladder through a submucosal tunnel. Finally a bilateral nephrectomy of the recipients own kidneys is performed.

2.3.3. Postoperative care

Penicillin (1 million I.U.) and streptomycin (1 g) daily is started at the time of the operation and continued for 5 days. Parenteral fluids (subcutaneous NaCl 0.9% and glucose 5%, or mixed infusions) are administered during the first two postoperative days. Thereafter a standard diet is given (Hope Farm). No immunosuppression is given and no special supportive measures are taken when rejection starts.

2.3.4. Determination of graft survival

Transplant function is assessed by regular estimations of the serum creatinine and urea. The postoperative day on which the animal died from renal insufficiency or on which the serum creatinine rose above 1000 μmole/l is taken as the end point of graft survival. The animals which remain alive with a creatinine above 1000 μ mole/l are killed with an overdose of pentothal. An autopsy and histological examination of the graft are always performed to check the diagnosis "immunological rejection".

Chapter 3 has been submitted for publication

CHAPTER 3

VALUE OF THE MIXED LYMPHOCYTE REACTION IN DOGS AS A GENETIC ASSAY

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Summary

For an optimal use of the mixed lymphocyte reaction (MLR) for genetical and transplantation research in dogs, insight into the kinetics of the test is required. Therefore, cell-dose response curves have been performed in various defined genetically different cell mixtures. The optimal cell dose was variable. No relation between genetic disparity and optimal cell doses or responder/stimulator cell ratios was found. In time-course kinetic studies earlier peak reactivity was observed with combinations of unrelated than of related dogs. No difference was found comparing one with two haplotype different related dogs. These results can be explained by the assumption that in those combinations which differ for the major histocompatibility complex (MHC), non-MHC gene products can influence the outcome of the MLR.

For a semi-quantitative evaluation a shorter culture period is preferred than that used for the determination of MLR negativity. Apparently the combined use of different modifications of the test procedure for a single cell combination may increase the power of the test as a genetic tool. It remains to be seen whether such modifications will be sufficient for a complete evaluation of the complex genetic control of the MLR and its relevance to homotransplantation.

Introduction

The major histocompatibility complex (MHC) is a chromosomal region, containing many different genes. Matching for antigens of the MHC in related donor-recipient pairs generally results in matching for whole MHC haplotypes and is an effective means to improve kidney allograft survival in such donor-recipient combinations (Singal et al., 1969; Brynger et al., 1977; Opelz and Terasaki et al., 1977). Matching for the whole MHC in unrelated donor-recipient pairs is practically impossible due the polymorphism of the genes which can be identified in the MHC and the far larger number of genes which cannot yet be identified.

At present it is unknown which loci of the MHC are most important for graft survival. Studies in mice have shown that some subregions of the MHC do contain transplantation genes, while others do not (Klein, 1975). Several clinical studies indicate that the outcome of the mixed lymphocyte reaction (MLR) is an important prognostic parameter for kidney allograft survival (Cochrum et al., 1973; Festenstein et al., 1976; Opelz and Terasaki, 1977).

For a precise determination of the importance of compatibility for MLR for homotransplant survival a detailed insight into the kinetics and the genetic control of the MLR is required. The dog appears to be a good animal model because of the knowledge which has been obtained in the past of it's MHC. The variety of possible immunogenetic different donor-recipient combinations encountered in man, is also encountered in the dog (Vriesendorp et al., 1977).

Previously, evidence has been reported which indicates that more than one pair of loci is involved in the genetic control of the MLR in the dog (Bijnen et al., 1977). In this report the applicability of the MLR as a genetic test is studied. Therefore the kinetics of the MLR and the correlation between the kinetics of the MLR and the genetic disparity between responder and stimulator cells has been investigated.

Material and methods

Mixed lymphocyte reactions

Blood was sampled from non-immunized dogs only. Lymphocyte preparation and incubation of cell mixtures was performed as previously described (Grosse-Wilde et al., 1973; Bijnen et al., 1977). In short, lymphocyte suspensions were prepared from heparinized blood on a Ficoll-Isopaque gradient. Each cell combination was tested in quadruplicate with the appropriate controls in microtiter plates. Stimulator cells were irradiated with 2500 rad. Culturing was done in medium RPMI-1640 at 38°C in a water-saturated atmosphere, containing 5% CO_2. Harvesting was done after a 16 hour period of labelling with tritiated

thymidine. The results are expressed in counts per minute as the mean of the quadruplicates.

Cell-dose response curves were obtained by varying the number of responder cells, keeping the number of stimulator cells constant (150,000 cells per well), by varying the number of stimulator cells, while keeping the number of responder cells constant (150,000 cells per well), and finally by varying the responder/stimulator cell ratio keeping the total number of cells constant (300,000 cells per well).

Time-course kinetics were studied by harvesting on days 5, 6, 7 and 8 of culture.

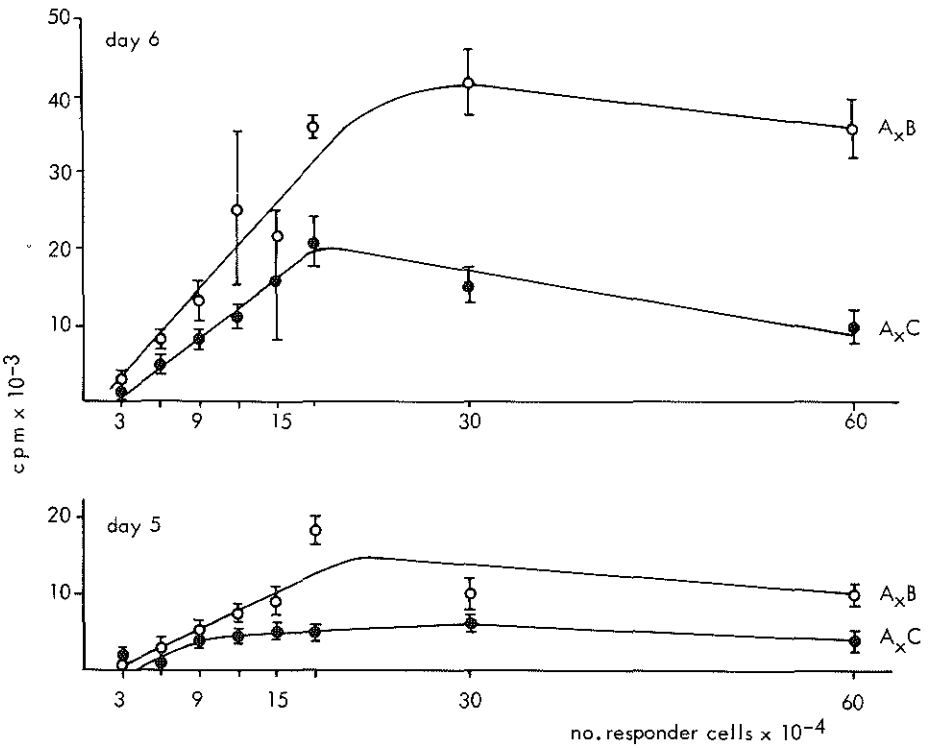

Figure 3.1. Optimal number of responder cells per well in MLR. The number of stimulator cells was 150,000 per well. Means ± standard error of the mean (s.e.m.) are given. All autologous controls were below 2,000 counts per minute (cpm).
● one haplotype difference
o two haplotypes difference

The experiments of figures 1,2,3,5 and 6 could not be repeated with the same cells for logistical reasons. However, similar curves were obtained in various other experiments with different cells of the same genetic combinations, except

for the stimulator cell-dose response curves (figure 2), of which only a small number of experiments was performed.

DLA typing

Typing for the DLA antigens (i.e. antigens of the MHC of the dog) was done by serological methods (the so called serologically defined or SD antigens) and lymphocyte cultures (the lymphocyte defined or LD antigens). DLA-SD typing was performed using techniques and antisera which were described previously (Vriesendorp et al., 1973; Vriesendorp et al., 1977). For DLA-LD typing the following homozygous reference cells were available during this study: DLA-50, DLA-51, DLA-52, DLA-53, DLA-54, DLA-55, DLA-56, DLA-57, DLA-58, R1 and R2. DLA and a number refer to the nomenclature recommendations which are given in the Joint Report of the Second Workshop on Canine Immunogenetics in Portland (1975). R1 and R2 refer to presently only locally (Rotterdam) recognized LD types, which await a more official designation after international comparison.

Cells from four genetically different combinations were analyzed in cell-dose response curves and time-course kinetics, namely DLA identical sibs, DLA one haplotype different sibs, DLA two haplotypes different sibs and DLA two haplotypes different unrelated dogs. Mongrels as well as beagles were used. DLA identity always included SD as well as LD identity; DLA differences were always for SD as well as for LD determinants.

Results

Cell dose response curves

Figure 1 shows the responder cell-dose response curve of a one and a two haplotype different combination on day 5 and day 6 of culture. The number of stimulator cells was fixed at 150,000 cells per well. The response appears to be proportional to the cell-dose up to a dose which is between 150,000 and 300,000 responder cells per well. The minimal responder cell dose which is required to obtain a positive result is between 30,000 and 60,000 cells per well. In some other tests this threshold cell-dose was even 100,000 cells per well. In this figure a maximal response is seen using 180,000 – 300,000 cells per well. With still higher responder cell-doses, a decrease of the response is noted.

More of such cell-dose response curves have been performed in different genetic combinations. Four different combinations of DLA identical siblings were tested with cell-doses up to 600,000 cells per well. The response was

always negative with any of the tested responder cell-doses. Furthermore, one haplotype different siblings, two haplotype different siblings and two haplotype different unrelated individuals have been tested. Table 1 shows that the optimal responder cell number is mostly equal in one and two haplotype different combinations. No differences are noted between the optimal responder cell-doses of unrelated compared to related individuals either.

Table 3.1. Optimal responder cell-dose and genetic disparity in MLR[a]. No obvious influence of MHC or non-MHC differences on the optimal responder cell-dose is observed.

	genetic relation between responder and stimulator		
responder	related 1 haplotype different	related 2 haplotypes different	unrelated 2 haplotypes different
1	1.2×10^5 ; 1.8×10^5	1.8×10^5	—
2	3.0×10^5	4.0×10^5	5.0×10^5
3	3.0×10^5	3.0×10^5	4.0×10^5
4	1.5×10^5	1.5×10^5 ; 1.5×10^5	—
5	3.0×10^5	3.0×10^5	1.5×10^5
6	3.0×10^5	1.8×10^5	1.5×10^5
7	3.0×10^5 ; 4.0×10^5	—	3.0×10^5

[a] Seven responder cells were tested in MLR with stimulator cells from one and two haplotypes different related and two haplotypes different unrelated cell donors. Harvesting was performed on the 5th day of culture. 150,000 stimulator cells were used per well. For responder cells 1, 3 and 7, cell-doses of 1, 2, 3, 4, 5 and 6 x 10^5 cells per well were tested. For responder cells 2, 4, 5 and 6, cell-doses of 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 3.0 and 6.0 x 10^5 cells per well were tested.
A comparison within one experiment can be made for the observed optimal responder cell-dose between the various genetic different culture combinations. The optimal responder cell-dose can be considered higher for the two haplotypes different (compared to the one haplotype different) related culture combinations in 2 instances (responder cells 1 and 2); equal in 3 instances (responder cells 3, 4 and 5) and lower in one instance (responder cell 6). Similarly the optimal responder cell-dose can be considered higher for unrelated (compared to related) culture combinations in 2 instances (responder cells 2 and 3), and lower in 3 instances (responder cells 5, 6 and 7). Thus, no obvious difference in optimal cell-dose between the various genetic groups is found.

Figure 2 shows a stimulator cell-dose response curve on day 5 and day 7 of culture. The number of responder cells was fixed at 150,000 cells per well. As with the responder cell-dose response curve, an optimal cell-dose can be seen. A positive reaction is seen with as few as 30,000 stimulator cells per well. In some other tests at least 60,000 stimulator cells per well were required to obtain a positive reaction. It is remarkable that with a culture period of 7 days the curves of the one and the two haplotypes different combinations in the figure cross each other. This test was performed only once; however it indicates that at a culture length of 7 days and 150,000 responder cells and 150,000 stimulator cells no

reliable quantitative interpretation can be made. At day 7 in this test, quantitative discrimination can only be made when 60,000 stimulator cells per well are used.

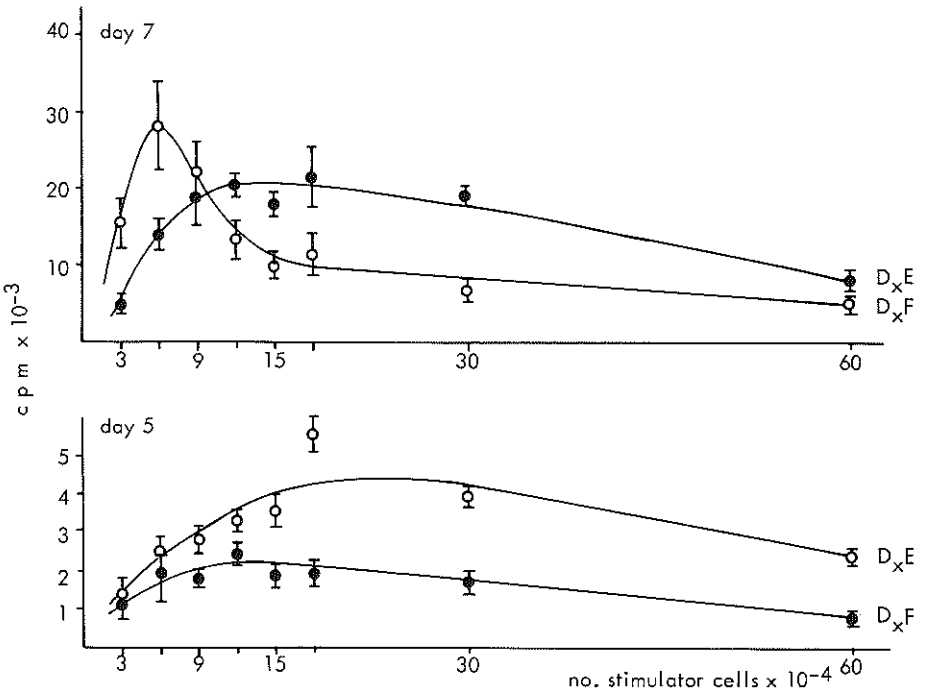

Figure 3.2. Optimal number of stimulator cells per well in MLR. The number of responder cells was 150,000 per well. Means ± s.e.m. are given. All autologous controls were below 2,000 cpm.
● one haplotype difference
o two haplotypes difference

Only a small number of stimulator cell-dose response curves was performed; therefore no conclusion can be drawn on the presence or absence of differences between the various genetic groups.

Figure 3 is an example of an experiment in which the influence of the responder/stimulator cell ratio was investigated, keeping the total number of cells constant (300,000 cells per well). The optimal responder/stimulator cell ratio appears to be variable. In 5 out of 12 experiments, peak reactivity was found at a 1/1 ratio. No obvious differences between the different genetic groups were noted. Negative reactions however were noted in 2 and 4 out of 12 mixtures when 2/1 and 5/1 responder/stimulator cell ratios were used respectively.

Thus, with responder/stimulator cell ratios above 1, false negative results may be obtained.

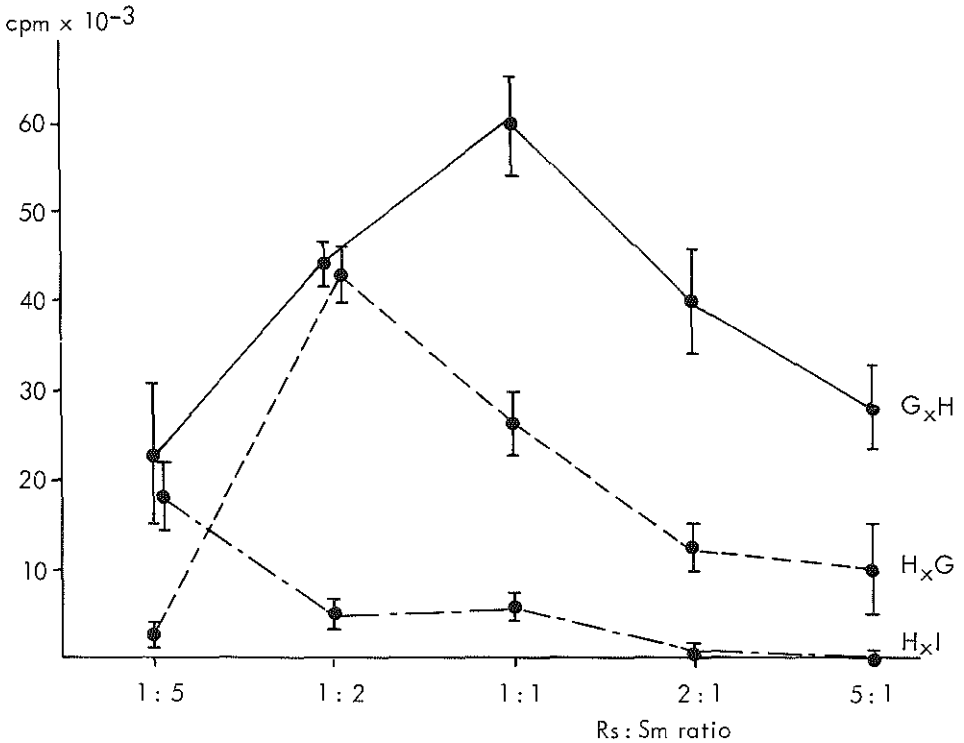

Figure 3.3. Optimal responder (Rs): stimulator (Sm) cell ratios in MLR. The total number of responder + stimulator cells was 300,000 per well. Means ± s.e.m. are given. All autologous controls were below 2,000 cpm.

Time – course kinetics

To determine the optimal day of harvest for a semiquantitative evaluation of the MLR, thirty different MLR mixtures have been harvested each on three different days of culture on two different occasions several weeks apart. Both experiments were performed with fresh cells. 150,000 responder cells and 150,000 stimulator cells per well were used. The results are presented in figure 4. The best correlation between the level of stimulation in the first and the second experiment was found when harvesting was performed on the 5th day of culture (figure 4[a], r = 0.78); the worst correlation was found when the mixtures were harvested on the 7th day of culture (figure 4[c], r = 0.44). Another experiment has been performed with similar results.

Furthermore, optimal stimulation may be obtained at different days of culture in different cell combinations. An example is shown in figure 5. Here, one responder cell reacts to different stimulator cells in one experiment. The peak reactivity of this responder cell is on day 5 (or maybe earlier), day 6 or day 7, depending on the stimulator cell used. Thus, the better test reproducibility on

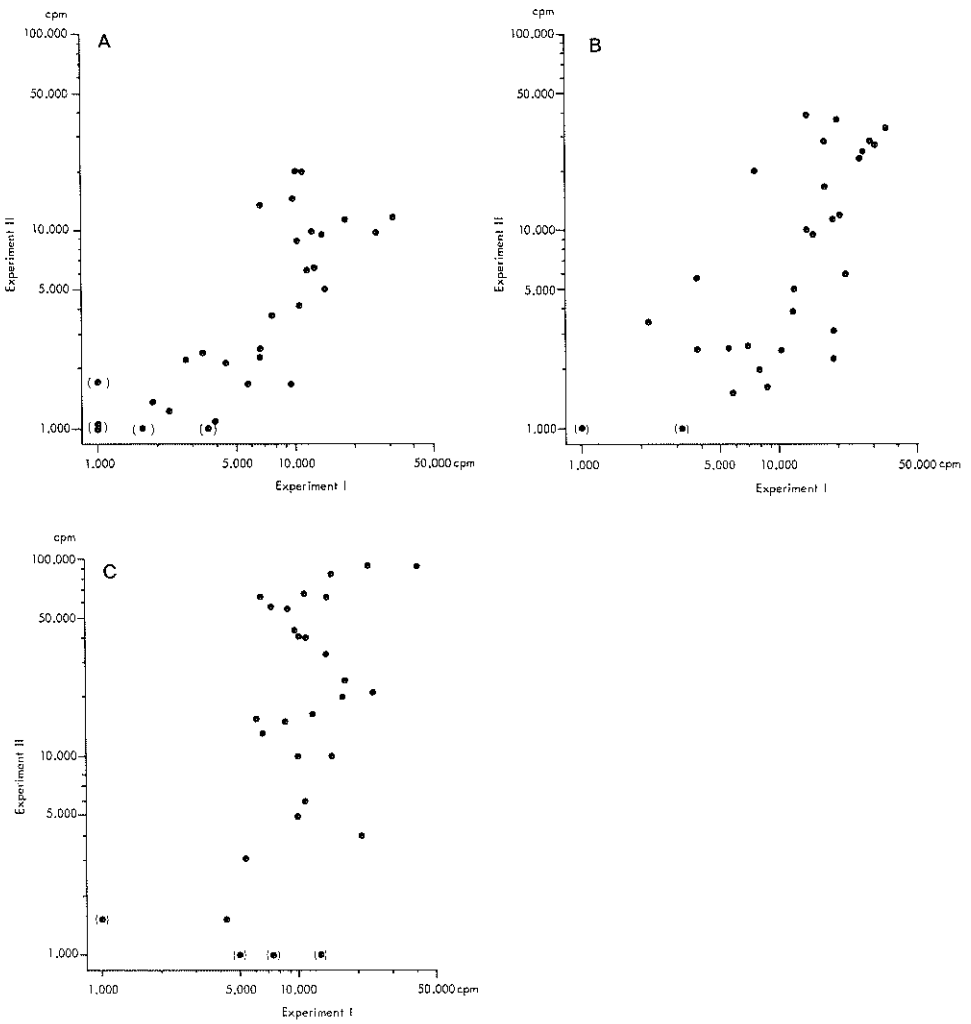

Figure 3.4. Optimal culture length and reproducibility of MLR. Thirty MHC different cell mixtures have been harvested each on day 5 (figure 4[a]), day 6 (figure 4[b]) and day 7 (figure 4[c]) of culture. The two combined experiments I and II were each performed with fresh cells with an interval of several weeks. The correlation coefficients were 0.78, 0.71 and 0.44 at day 5, 6 and 7 of culture respectively (Spearman test). Only the means of the quadruplicates are given. All autologous controls were below 2,000 cpm.
(●) means: cpm $<$1,000

day 5 of culture and the differences in time-course kinetics both imply that early (day 5) harvesting is essential when a semi-quantitative evaluation of the MLR is needed. In some tests however peak reactivity was seen on day 8 or

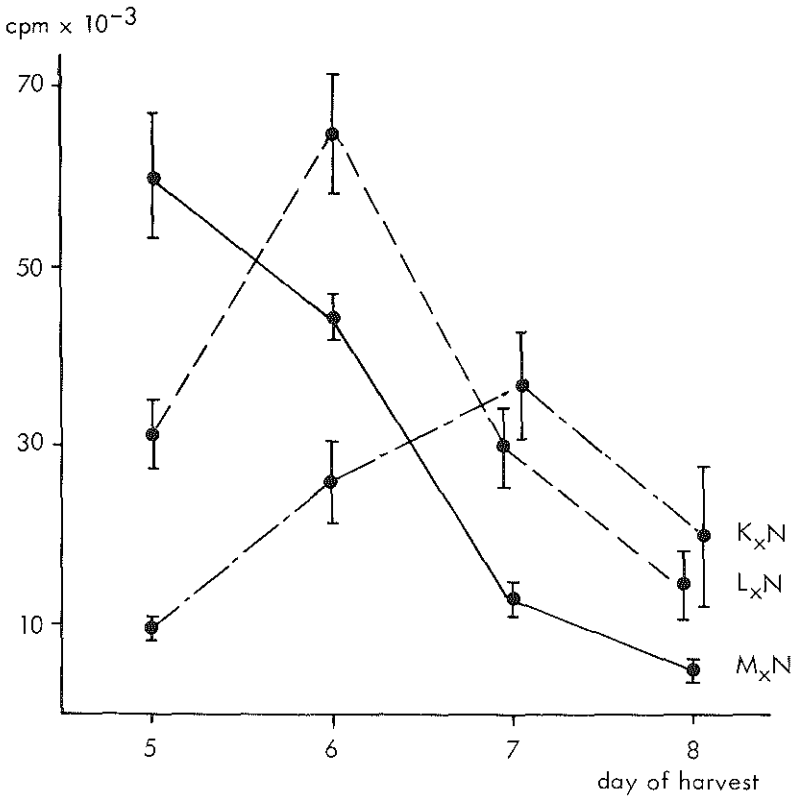

Figure 3.5. Optimal harvest day in MLR. 150,000 responder cells and 150,000 stimulator cells per well were used. Means ± s.e.m. are given. All autologous controls were below 2,000 cpm. These curves were obtained by testing one responder cell against three different stimulator cells in one experiment.

later, while no significant reactivity was seen on day 5. Therefore it appears advisable for the establishment of MLR negativity to harvest on a later day than the 5th day of culture.

Time-course kinetics have also been performed with different responder/stimulator cell ratios. An example is shown in figure 6. It appears that the day of peak reactivity here is independent of the cell ratios used. Thus it is unlikely that exhaustion of the culture medium is an important cause for the observed decline in reactivity after a certain number of days in culture. The results suggest rather that the day of peak reactivity is dependent on the tested cell combinations. Possibly, genetic factors play a role. Therefore, time-course kinetics have been

performed in different combinations. Table 2 presents the results. It appears that peak reactivity is more variable and generally earlier in the unrelated compared to the related MLR combinations ($\chi^2 = 7.18$, $P < 0.05$). No difference in peak

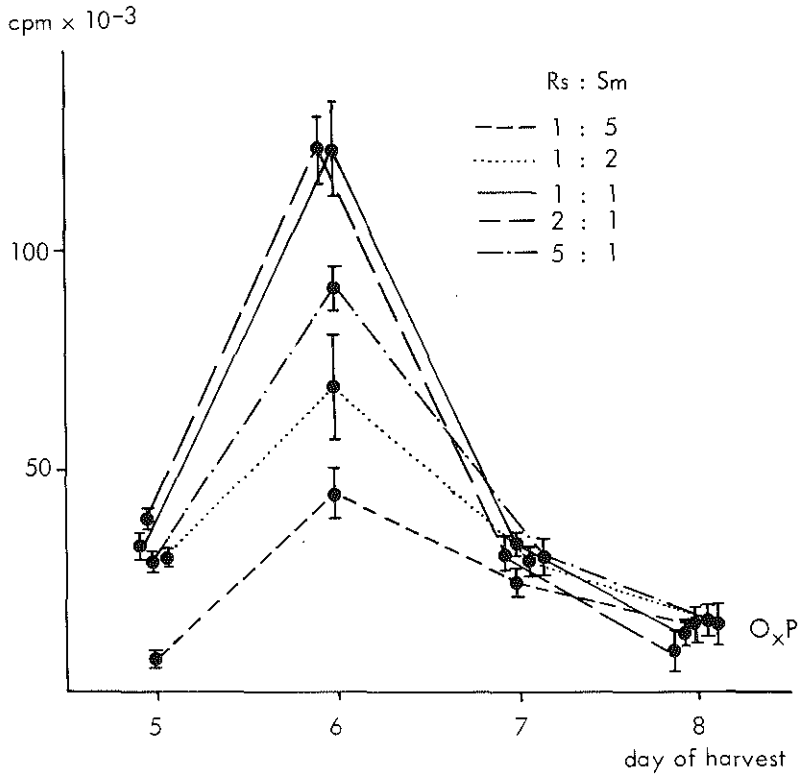

Figure 3.6. Optimal responder (Rs): stimulator (Sm) ratio and harvest day in MLR. The total number of responder + stimulator cells was 300,000 per well. Means ± s.e.m. are given. All autologous controls were below 2,000 cpm. The curves were obtained by testing one responder - stimulator cell combination in different ratios in one experiment.

day has been observed between one and two haplotype different related combinations. These observations suggest that non-MHC genes influence the outcome of the MLR. However, no stimulation has been seen at any length of culture in six DLA identical sibling combinations. Apparently, the role of non-MHC genes can probably only be appreciated in MHC different combinations.

Discussion

The kinetics of the MLR in dogs have been described before by Goldmann and Flad (1975) for a semi-micromethod in small test tubes. The present results in

Table 3.2. Optimal culture length in days and genetic disparity in MLR[a]. No obvious influence of MHC differences on the optimal culture length is observed, but non-MHC differences may accelerate (or delay) the response

	genetic relation between responder and stimulator		
responder	related 1 haplotype different	related 2 haplotypes different	unrelated 2 haplotypes different
1	6 ; 6	6	5
2	6 ; 6	6	5
3	7	7	6
4	6 ; 6 ; 6 ; 7	-	5
5	8 ; 8	-	6 ; 7
6	6 ; 6 ; 6 ; 6	-	6
7	7	7	7
8	6 ; 6	6 ; 6	-
9	-	6	6 ; 7 ; 7
10	-	6	7

[a] Ten responder cells were tested in MLR with stimulator cells from one and two haplotypes different related and two haplotypes different unrelated cell donors. Harvesting was performed on the 5th, 6th, 7th and 8th day of culture. 150,000 responder cells and 150,000 stimulator cells per well were used. The optimal culture days are given.
A comparison of the optimal culture length in days within one experiment can be made between the various genetic different culture combinations. No difference in culture length was observed between the 5 one and two haplotype different related culture combinations tested (responder cells 1, 2, 3, 7 and 8). The optimal culture length in two haplotypes different unrelated culture combinations was shorter compared to two haplotypes related culture combinations in 3 instances (responder cells 1, 2 and 3) and compared to one haplotype different related culture combinations in 2 instances (responder cells 4 and 5); equal to a one and a two haplotypes different combination each in 1 instance (responder cells 6 and 7 respectively); and longer compared to two haplotypes different culture combinations in 2 instances (responder cells 9 and 10). Thus, the optimal culture length appears to be more variable (and mostly shorter) in unrelated compared to related culture combinations.

microtiter plates confirm and extend their results. It appears that relatively high numbers of responder and stimulator cells (over 100,000 per well each) in a ratio of 1/1 can safely be used in order to determine whether an MLR between two individuals is positive or negative. This means that the procedure routinely used in our laboratory (150,000 responder cells and 150,000 stimulator cells, harvest on the 6th day of culture) is applicable for a determination of MLR negativity and MLR positivity. However, no quantitative conclusions can be drawn. For a semi-quantitative comparison of the reactivity of one responder cell against several different stimulator cells in the same experiment, it would appear better to use lower numbers of responder and stimulator cells (e.g. 100,000 cells per well each) and to harvest earlier (day 4 or 5). With such a procedure false negative results may be obtained more often, but quantitative differences can be measured which would be obscured when testing occurred beyond the peak of the MLR reactivity curve.

Important prerequisites for a test which is used to assign LD determinants to individuals, are that Mendelian inheritance of the determinants can be shown and that their segregation in a family should predict for negative and positive MLR's within that family. This is usually the case (Bijnen et al., 1977 - Chapter 4; Vriesendorp et al., 1977). However an exception to this rule has been described (Bijnen et al., 1977 - Chapter 4). In contrast, if two unrelated dogs have two different LD determinants in common, the MLR is often positive (Bijnen et al., 1977 - Chapter 4). The described discrepancies between LD typing and the outcome of the direct MLR may be explained by assuming more than one locus for MLR. An alternative explanation is that with the LD typing cells used, not only LD identical individuals are selected, but also individuals with slightly different but "cross-reacting" LD antigens. In this report evidence has been obtained which suggests that differences for non-MHC genes can amplify the effect of differences for MHC genes. If this hypothesis were to prove true, then it would mean that small MHC differences may sometimes result in a strongly positive MLR, obscuring MHC similarity. Thus positive and, in particular, weakly positive MLR reactions (as the so called "typing responses") in man (Keuning et al., 1975) might be indicative of differences between stimulator and responder cells for MHC gene products as well as for non-MHC gene products.

Clearly a better insight into the genetic control of the MLR is required for a more exact evaluation of the genetics and histocompatibility effect of the individual LD loci. A further combined use of different modifications of the MLR for each test combination in new family and population studies will show whether such modifications sufficiently increase the power of the MLR as a genetic tool. If they do not allow an adequate discrimination between MHC and non-MHC influences on MLR reactivity, other methods may be required for the determination of LD gene products.

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BRYNGER, H., SANDBERG, L., AHLMEN, J., BITTER-SUERMANN, H., BLOHME, I., LINDHOLM, A., & GELIN, L.E.(1977) Effect of HLA compatibility on primary kidney grafts from living related and cadaveric donors. Transplant. Proc. 9, 479-482

BIJNEN, A.B., VRIESENDORP, H.M., GROSSE-WILDE, H., & WESTBROEK, D.L. (1977) Polygenic control of mixed lymphocyte reactions in dogs. Tissue Antigens, 9, 187-194

COCHRUM, K.C., PERKINS, H.A., PAYNE, R.O., KOUNTZ, S.L. & BELZER, F.O. (1973) The correlation of MLR with graft survival. Transplant. Proc. 5, 391-396

FESTENSTEIN, H., SACHS, J.A., PARIS, A.M.I., PEGRUM, G.D., & MOORHEAD, J.F. (1976) Influence of HLA matching and blood transfusion on outcome of 502 London transplant group renal graft recipients. Lancet, i, 157-161

GOLDMANN, S.F., & FLAD, H.D. (1975) Histocompatibility testing in dogs I. A semi-micro mixed lymphocyte culture (MLC) technique for histocompatibility matching in dogs. Tissue Antigens 5, 145-154

GROSSE-WILDE, H., BAUMANN, P., NETZEL, B., KOLB, H.J., WANK, R., MEMPEL, W. & ALBERT, E.D. (1973) One way non-stimulation in MLC related to DL-A homozygosity. Transplant. Proc. 5, 1567-1571

JOINT REPORT OF SECOND INTERNATIONAL WORKSHOP ON CANINE IMMUNOGENETICS (1976) Transplant.Proc. 8, 289-311

KEUNING, J.J., TERMIJTELEN, A., BLUSSE VAN OUD ALBLAS, A., GABB, B.W., D' AMARO, J.& VAN ROOD, J.J. (1975) LD (MLC) population and family studies in a Dutch population. Histocompatibility Testing 1975, 533-543

KLEIN, J. (1975) Biology of the mouse histocompatibility-2 complex. Springer-Verlag, New York

OPELZ, G. & TERASAKI, P.I. (1977[a]) Significance of mixed leukocyte culture testing in cadaver kidney transplantation. Transplantation 23, 375-380

OPELZ, G. & TERASAKI, P.I., (1977[b]) Studies on the strength of HLA antigens in related donor kidney transplants. Transplantation 24, 106-111

SINGAL, D.P., MICKEY, M.R. & TERASAKI, P.I. (1969) Serotyping for homotransplantation XXIII. Analysis of kidney transplants from parental versus sibling donors. Transplantation 7, 246-258

SOLHEIM, B.G., FLATMARK, A., ENGER, E., HEWELL, J. & THORSBY, E. (1977) Influence of HLA-A, -B, -C and -D matching on the outcome of clinical kidney transplantation. Transplant. Proc. 9, 475-478

VRIESENDORP, H.M. (1973) The major histocompatibility complex of the dog. Thesis, Erasmus University, Rotterdam, the Netherlands. Bronder Offset, Rotterdam, the Netherlands

VRIESENDORP, H.M., GROSSE-WILDE, H., & DORF, M.E. (1977) The major histocompatibility complex of the dog. In Götze, D. (ed): The major histocompatibility system in man and animals. Springer- Heidelberg, Germany, chap. 3, p. 129-163

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CHAPTER 4

POLYGENIC CONTROL OF MIXED LYMPHOCYTE REACTIONS IN DOGS

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Summary

The genetics of the MLR of the dog are studied using LD typing cells. The four new homozygous reference cells described bring the total number of defined LD specificities in this species to 11. In families, LD specificities generally segregate en bloc with the SD specificities of the DLA complex. The recombination frequency between the SD and LD loci is provisionally estimated to be 0.007. A slight but significant stimulation between two SD identical LD typed siblings could not be explained on the basis of a single recombination between the SD loci and one LD locus and therefore suggested the existence of an additional LD system.

Unrelated SD different individuals with two different LD determinants in common frequently stimulate each other in MLR, but low responses are also encountered. The results described are compatible with a hypothesis of two segregant series of alleles for the genetic control of the MLR and linkage disequilibrium between certain alleles of these series. More conclusive evidence of the validity of this hypothesis and further knowledge of other factors which determine the response in the MLR are required before a reliable estimate of the importance of LD structures in histocompatibility can be made.

Introduction

Histocompatibility studies in the dog have shown that, as in other species, one Major Histocompatibility Complex (MHC) exists, which is located on an autosomal chromosome (Vriesendorp et al. 1971, Templeton & Thomas 1971, Joint Report of the First International Workshop on Canine Immunogenetics 1973, Joint Report of the Second International Workshop on Canine Immunogenetics, 1976).

The genetic control of lymphocyte defined (LD) antigens which determine the outcome of Mixed Lymphocyte Reactions (MLR) was found to be closely linked to, but distinct from, the genetic control of serologically defined (SD) antigens (van den Tweel et al. 1974 , Grosse-Wilde et al. 1974). Typing for LD determinants in dogs (Grosse-Wilde et al. 1975, Goldmann et al. 1975) can be performed following the same principles as in man (Mempel et al. 1973, Keuning et al., 1975[a]), i.e. by using LD homozygous cells as stimulators in MLR. For a determination of the relevance of LD typing to histocompatibility in unrelated donor-recipient pairs, detailed information on the genetic control of the MLR is required.

Although, in the past, the majority of canine MLR results have been explained by assuming the presence of one locus only for MLR, evidence has been obtained indicating that more loci might be involved (Grosse-Wilde et al.1975[b]). In this report new family and population studies are presented which are most efficiently explained by a polygenic control of MLR in the dog.

Material and Methods

Dogs

Blood samples were taken from beagles, labradors and mongrel dogs of the colonies of Het Centraal Proefdierenbedrijf TNO, Austerlitz, the Netherlands (Dr. J. van Vliet) and of the Erasmus University, Rotterdam (Dr. W. van Dijk).

Serology

A battery of 70-90 different alloantisera was used in a one-stage microcytotoxicity test to type for the serologically defined antigens as described before (Vriesendorp 1973, Smid-Mercx et al. 1975).

Mixed lymphocyte reactions

Unilateral cultures were performed in a micromethod as described previously for

the dog (Grosse-Wilde et al. 1973). In brief, lymphocyte suspensions were prepared from heparinized blood by centrifugation on a Ficoll-Isopaque gradient. Each cell combination was tested fourfold with the appropriate controls in tilted flat bottom microtiter plates, using 150,000 responder cells and 150,000 stimulator cells per well. Stimulator cell suspensions were irradiated with 2500 rad. Culturing was done in RPMI-1640 at 38°C in a water-saturated atmosphere, containing 5% CO_2. Harvesting was performed on the sixth day of culture after a 16-h period of labelling with tritium-labelled thymidine. The results are expressed as the mean of the quadruplicates, or as a relative response (*R.R.*), i.e. the percentage of the median of the three highest responses of a given cell.

Other markers

Polymorphic red and white cell enzymes (Meera Khan et al. 1973) and canine secretory alloantigens (Zweibaum et al. 1974) were tested to check the reliability of the recorded paternity.

Nomenclature

The new recommendations for the nomenclature of the loci within the MHC in the dog are used (Joint Report of the Second International Workshop on Canine Immunogenetics 1976). In analogy with the situation in man, the SD loci have been labelled DLA-A and DLA-B. A third SD locus, which has recently been recognized (Vriesendorp et al. 1977) and provisionally has been designated DLA-C, is not included in this analysis.

The LD systems will remain unlabelled until more data are available on the number of LD loci within the DLA complex and the assignment of currently known LD specificities to these loci.

Results

LD typing cells

At present 11 different homozygous reference cells are available (Table 1). They all stimulate each other in MLR and hence represent different specificities. For technical reasons the specificity R2, associated with DLA-A7-B- in the beagle was not tested with DLA-54. As the specificity DLA-54 was not present in eight beagles carrying the haplotype DLA-A7-B- (Grosse-Wilde et al., 1975b), R2 and DLA-54 probably represent different LD types. The old specificities DLA-50, DLA-51, DLA-52, DLA-53, DLA-54, DLA-55 and DLA-56 have been described before (Grosse-Wilde et al., 1975b). The new specificities have

Table 4.1. MLR results expressed in cpm x 10^{-3} of 11 LD homozygous typing cells

Dog	LD type	SD type	50_x	51_x	52_x	53_x	54_x	55_x	56_x	57_x	58_x	$R1_x$	$R2_x$
4.440	50	A2 -B4 /A2-B4	0	50	57	NT[a]	7	72	59	26	48	29	14
D2.179	51	A2 -B5 /A2-B5	27	0	74	NT[a]	8	73	59	21	24	39	37
D4.583	52	A9 -B6 /A9-B6	42	46	0	NT[a]	12	55	47	24	34	30	20
B 270	53	A3 -B—/A3-B—	12	27	48	0	6	38	43	23	33	20	9
017	54	A10-B5 /A7-B—	6	7	8	NT[a]	0	13	13	6	10	29	NT[a]
066	55	A3 -B—/A3-B—	28	59	46	23	10	0	52	24	31	22	61
L 16	56	A1 -B13/A1-B13	38	50	44	41	8	41	2	23	44	13	56
37 WX	57	A8 -B13/A8-B13	18	28	22	19	16	20	20	0	18	9	25
4.334	58	A9 -B4 /A9-B4	24	39	58	60	17	64	21	43	0	22	32
T 46	R1	A7 -B13/A7-B13	16	14	13	15	17	20	20	9	32	0	16
D5.314	R2	A7 -B—/A7-B—	30	67	NT[a]	65	NT[a]	75	43	38	46	29	0

[a] NT = not tested. Difference of LD type is known from earlier experiments

Table 4.2. MLR results of a dog family (expressed as a relative response) favoring the existence of two loci for LD

	SD genotype	F_x	M_x	1_x	2_x	3_x	4_x	5_x	6_x	50_x	52_x	56_x	58_x	R.R. = 100 % cpm x 10^{-3}
Father	A9-B6/A9-B4	2	56	84	56	64	164	100	100	88	4	80	8	25
Mother	A2-B4/A1-B13	89	8	78	98	67	100	78	83	16	78	6	100	18
Sib 1	A9-B6/A2-B4	81	45	2	1	63	151	100	89	2	1	72	96	57
Sib 2	A9-B6/A2-B4	66	60	2	7	78	107	82	96	10	7	100	93	28
Sib 3	A9-B6/A2-B4	67	67	59	133	2	100	89	93	11	85	70	7	27
Sib 4	A9-B6/A1-B13	45	33	51	109	73	1	1	18	70	3	2	100	33
Sib 5	A9-B6/A1-B13	75	92	108	100	100	1	1	15	55	3	3	100	30
Sib 6	A9-B6/A1-B13	61	56	89	117	100	14	16	2	82	3	31	83	36

provisionally been labelled DLA-57 (Munich), DLA-58 (Rotterdam), R1 (Rotterdam) and R2 (Rotterdam). The homozygosity of all typing cells has been been verified by a one-way non-stimulation with *both* parental cells with the exception of DLA-55 which was only non-stimulatory to paternal cells.

LD-SD inheritance in dog families

Forty-nine members of six different beagle families were tested in a family MLR together with the relevant typing cells. In none of the families was false paternity observed. As a rule, an LD determinant segregated en bloc with a particular SD haplotype. In all families LD typing of the offspring predicted the outcome of the MLR performed between sibs. Thus sibs with two LD specificities in common did not stimulate each other in MLR, while the MLR in combinations with different LD specificities was positive. In one family two exceptions were observed (Table 2). In six individuals the SD haplotype A9-B6 of the father was associated with the LD determinant DLA-52. Sib 3 inherited the SD haplotype A9-B6 together with the paternal LD determinant DLA-58. Thus a recombination between SD and LD must have occurred. This is consistent with the results of the family MLR, as sib 3 is positive in MLR with its SD identical sibs 1 and 2. Sib 6 differed slightly in MLR from her SD identical sisters 4 and 5. This observation cannot be explained by assuming a simple recombination between SD and LD, as she strongly differs in MLR from her brothers 1 and 2. The LD typing results are consistent, as she reacts slightly to the homozygous typing cell DLA-56.

Repeated MLR testing and SD typing confirmed the results in this family.

Recombination frequency

In our total family material, 241 haplotypes were informative for a recombination between SD and LD. In one haplotype, a recombination was observed. If these data are combined with earlier obtained family MLR data (van den Tweel et al. 1974) the recombination frequency between the SD and the major LD locus in the dog can be estimated to be 2:307 = 0.007 (5% confidence limits 0.001-0.024).

LD typing in unrelated individuals

In Fig. 1, the MLR results of 46 SD different heterozygous unrelated cell combinations (mongrel-mongrel and beagle-mongrel) with two LD specificities in common (as defined by previous LD typing by Grosse-Wilde et al. 1975[b]) are shown. It can be concluded that the MLR is frequently positive. A low response (R.R. below 30%) is observed in 26% of the tested combinations. The R.R. in

this group is significantly lower than the R.R. in 50 SD different LD different combinations ($P < 0.001$, Wilcoxons rank sum test).

The level of the R.R. was not related to the LD specificities in common. No apparent correlation was observed between a higher R.R. and the presence of differences for antigens of the DLA-A and/or DLA-B series in this limited material.

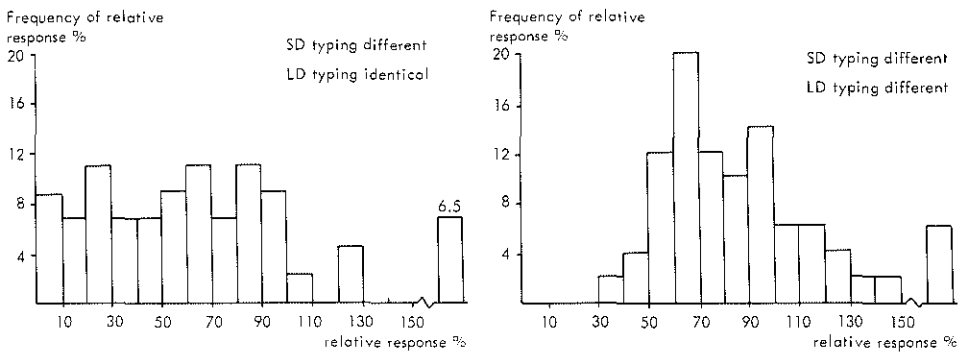

Figure 4.1. Relative responses in 46 SD different combinations with two LD determinants in common ("LD typing identical") and 50 SD different LD different combinations.

Discussion

Negative MLR's are rare between random unrelated individuals. This limits the evaluation of the influence of a negative MLR on graft survival. Typing for LD may be a tool to select donor-recipient pairs that will not stimulate each other in MLR. The four new canine LD homozygous reference cells described here bring the number of different defined LD specificities in this species to 11. An estimate of the remaining, still undefined LD alleles cannot be made until the number of different LD loci is known.

The reported results of LD typing in dog families demonstrate that LD specificities are genetically determined characteristics which segregate en bloc with the SD specificities of the DLA complex. The observation that sibs which are identical for two independent LD specificities never stimulate each other in MLR, while sibs, which differ for one or two LD specificities, always do, even in the event of a recombination between the SD and LD loci (Table 2), strongly suggests that by LD typing, one types for structures that determine the outcome of the MLR.

The recombination frequency between the SD and LD loci of 0.007 in the dog is similar to the recombination frequency in man (0.007, Keuning et

al. 1975b; 0.009, Netzel et al. 1975). These calculations are based on the assumption that only one major MLR locus exists. A more accurate figure cannot be given before the number and relative positions of the SD and LD loci are known.

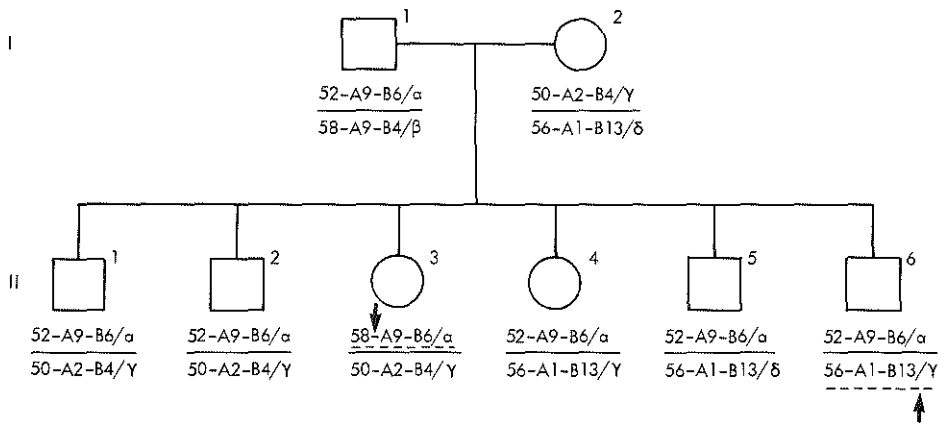

Figure 4.2. Proposed genotypes in an SD-LD recombination family. Genes of an additional LD locus are represented by Greek characters.

The occurrence of a weak but significant stimulation between sib 6 and its SD identical sibs 4 and 5 of table 2 coincides with a low but significant response to the typing cell DLA-56. This can be explained by a polygenic control of the MLR. The proposed genotypes of this family are depicted in Fig. 2. The determinants of the new LD locus are symbolized by Greek characters. They may be identical in the father, but not in the mother. This new LD locus may or may not be situated on the same chromosome carrying the DLA complex, but should be located outside DLA-A and B.

The results in this dog family are very similar to the human family described by Suciu-Foca & Dausset (1975). Less obvious explanations for the observations in this family are the possibility of a mutation in an LD gene or a double recombination in the MHC region of one chromosome.

From earlier studies in the dog it is known that a high linkage disequilibrium exists between certain alleles of the SD and LD loci (van den Tweel et al. 1974). Therefore, a negative MLR is frequently found between SD identical individuals, but rarely between SD different dogs. From the results with typing cells in families one might expect that the MLR between SD different unrelated dogs with two LD determinants in common is negative. However, it now appears that the MLR is frequently positive. This observation favors the hypothesis that more than one LD locus exists. On the other hand, the finding that in 26% the R.R. is below 30% indicates that with incomplete LD typing, identity may occur due to

the presence of linkage disequilibrium between LD alleles of different series. This situation is similar to the situation in man, where a positive MLR was also frequently found between persons identical for two LD specificities (Grosse-Wilde et al., 1975[a]).

A considerable number of observations is now available which argue against the genetic control of the MLR in the dog by only one locus, i.e.:

a) the occurrence of more than one LD determinant in a typing cell and a haplotype (Grosse-Wilde et al., 1975[b]).
b) a positive reaction between SD identical LD typed siblings, which cannot be explained by a single recombination between the SD loci and only one LD locus (this report).
c) a slightly positive reaction in MLR of some individuals of a family with only one out of several mutually negative homozygous cells of the same family (Goldmann et al., 1975).
d) a high frequency of positive MLR's between unrelated individuals with two LD determinants in common (this report).
e) variations of culture lengths suggest the influence of additional genetic systems on MLR (chapter 3).

Therefore, in dogs as well as in mice (Meo et al. 1973) and in men (Mempel et al. 1973, Suciu-Foca & Dausset 1975, Bijnen et al. 1976) the available evidence indicates a polygenic control of the MLR. If this new model is valid, each LD homozygous reference cell should be submitted to a specificity analysis, since homozygosity can be present for only one or more of the LD loci involved. It then follows that it is important to determine whether a typing cell is homozygous by descent or not, as homozygosity by descent automatically implies homozygosity for all LD loci of the MHC. Phenomena described as "inclusion", "crossreactivity" and "typing response" in LD typing can easily be explained by a multiple locus model. A further definition of the genetics and polymorphism in the control of the MLR will allow for a better identification of LD identical unrelated individuals, facilitating the analysis of the possible histocompatibility effect of LD loci in a preclinical model such as the dog.

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References

BIJNEN, A.B., SCHREUDER, I., VOLKERS, W.S., PARLEVLIET, J. & VAN ROOD, J.J. (1977) The lymphocyte activating influence of the HL-A region. J. Immunogen. 4, 1-5

GOLDMANN, S.F., KRUMBACHER, K., SCHNAPPAUF, H. & FLAD, H.D. (1975) Definition of MLC specificities in the dog. Transplant. Proc. 7, 389-393

GOLDMANN, S.F., KRUMBACHER, K., SCHNAPPAUF, H.P., HUGET, R.P. & FLAD, H.D. (1975) Histocompatibility testing in dogs. II. Leukocyte typing in relation to the mixed lymphocyte culture (MLC) reactivity. Tissue Antigens 5, 155-164

GROSSE-WILDE, H., BAUMANN, P., NETZEL, B., KOLB, H.J., WANK, R., MEMPEL, W. & ALBERT, E.D. (1973) One way non stimulation in MLC related to DL-A homozygosity. Transplant. Proc. 5, 1567-1571

GROSSE-WILDE, H., VRIESENDORP, H.M., WANK, R., MEMPEL, W., DECHAMPS, B., HONAUER, U., BAUMANN, P., NETZEL, B., KOLB, H.J. & ALBERT, E.D. (1974) Identification of four MLC specificities in the dog. Tissue Antigens 4, 229-237

GROSSE-WILDE, H., NETZEL, B., RUPPELT, W., BREHM, G., BERTRAMS, J., EWALD, R., LENHARD, V., RITTNER, CH., SCHOLZ, S. & ALBERT, E.D. (1975[a]) Immunogenetics of LD determinants in man. Histocompatibility Testing 1975, pp. 526-531 Munksgaard, Copenhagen

GROSSE-WILDE, H., VRIESENDORP, H.M., NETZEL, B., MEMPEL, W., KOLB, H.J., WANK, R. & ALBERT, E.D. (1975[b]) Immunogenetics of seven LD alleles of the DLA complex in mongrels, beagles and labradors. Transplant. Proc. 7, 159-164

JOINT REPORT FIRST INTERNATIONAL WORPSHOP ON CANINE IMMUNOGENETICS (1973) Tissue Antigens 3, 145-163

JOINT REPORT SECOND INTERNATIONAL WORKSHOP ON CANINE IMMUNOGENETICS (1976) Transplant. Proc. 8, 289-311

KEUNING, J.J., TERMIJTELEN, A., BLUSSE VAN OUD ALBLAS, A., GABB, B.W., AMARO, J.D. & VAN ROOD, J.J. (1975a) LD (MLC) population and family studies in a Dutch population. Histocompatibility Testing 1975, pp. 533-543 Munksgaard, Copenhagen

KEUNING, J.J., VAN DEN TWEEL, J.G., GABB, B.W., TERMIJTELEN, A., GOULMY, E., BLOKLAND, E., ELFERINK, B.G. & VAN ROOD, J.J. (1975b) An estimation of the recombination fraction between the MLC locus and the FOUR locus. Tissue Antigens 6, 107-115

MEERA KHAN, P., LOS, W.R.T., VAN DER DOES, J.A. & EPSTEIN, R.B. (1973) Iso enzyme markers in dog blood cells. Transplantation 15, 624-628

MEMPEL, W., GROSSE-WILDE, H., ALBERT, E. & THIERFELDER, S. (1973) Atypical MLC reactions in HL-A typed related and unrelated pairs. Transplant. Proc. 5, 401-408

MEMPEL, W., GROSSE-WILDE, H., NETZEL, B., LUBOLDT, W., BERTRAM, J. & ALBERT, E.D. (1975) Definition and population studies of LD determinants in man. Transplant. Proc. 7, 81-85

MEO, T., VIVES, J., MIGGIANO, O. & SHREFFLER, D. (1973) A major role for the Ir-1 region of the mouse H-2 complex in the mixed leukocyte reaction. Transplant. Proc. 5, 377-381

NETZEL, B., GROSSE-WILDE, H., RITTNER, CH., PRETORIUS, A.M.G., SCHOLZ, G. & ALBERT, E.D. (1975) HL-A/MLC recombination frequency and LD typing in HL-A/MLC/Bf/PGM$_3$ recombinant families. Histocompatibility Testing 1975, pp. 955-959 Munksgaard, Copenhagen

SMID-MERCX, B.M.J., DUYZER-DEN HARTOG, B., VISSER, T.P. & VRIESENDORP, H.M. (1975) Serological studies on canine histocompatibility antigens. Transplant. Proc. 7, 361-364

SUCIU-FOCA, N. & DAUSSET, J. (1975) Complex MLR family patterns. Immunogenetics 2, 389-391

TEMPLETON, J.W. & THOMAS, E.D. (1971) Evidence for a major histocompatibility locus in the dog. Transplantation 11, 429-431

TWEEL, J.G. VAN DEN, VRIESENDORP, H.M., TERMIJTELEN, A., WESTBROEK, D.L., BACH, M.L. & VAN ROOD, J.J. (1974) Genetic aspects of canine mixed lymphocyte cultures. J. exp. Med. 140, 825-836

VRIESENDORP, H.M. (1973) Major histocompatibility complex of the dog. Thesis, Erasmus University Rotterdam. Bronder Offset, Rotterdam

VRIESENDORP, H.M., BIJNEN, A.B., WESTBROEK, D.L. & VAN BEKKUM, D.W. (1977) Genetics and transplantation immunology of the DLA complex. Transplant. Proc. 9, 293-296

VRIESENDORP, H.M., ROTHENGATTER, C., BOS, E., WESTBROEK, D.L. & VAN ROOD, J.J. (1971) The production and evaluation of dog allolymphocytotoxins for donor selection in transplantation experiments. Transplantation 11, 440-445

ZWEIBAUM, A., ORIOL, R., FEINGOLD, N., DUSSAULT, E., ROUSSET, M. & CHEVALIER, G. (1974) Studies on canine secretory alloantigens (CSA). Tissue Antigens 4, 115-129

Chapter 5 has been submitted for publication

CHAPTER 5

GENETICS OF KIDNEY ALLOGRAFT SURVIVAL IN DOGS

I Relevance of minor histocompatibility systems in recipients without immunosuppressive therapy

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Summary

To evaluate the importance of antigens not belonging to the canine major histocompatibility complex (MHC), the DLA complex, for renal allograft survival, kidneys were transplanted in various genetically different donor-recipient combinations. No immunosuppressive therapy was given. Histocompatibility testing was performed by serology alone or in combination with mixed lymphocyte reactions (MLR's) in beagles and by serological typing and MLR's in mongrels.

Renal allograft survival in DLA matched non-sibling beagles was significantly shorter than in DLA matched littermate beagles. A larger number of differences for non-DLA antigens in non-siblings compared to littermates is probably responsible for the discrepancy in survival times. The cumulative effect of these non-DLA antigens is comparable in strength to the DLA antigens, as the survival times in the matched non-siblings were close to the survival times in one haplotype mismatched littermates.

Kidney allograft survival in DLA matched unrelated mongrels was still shorter than in DLA matched non-sibling beagles, possibly due to a larger heterogeneity of non-DLA antigens in mongrels. MLR and/or serologically matched beagles and MLR matched mongrels always survive significantly longer than MLR and serologically mismatched mongrels.

The number and polymorphism of non-DLA systems, relevant for kidney allograft survival was calculated to be limited in beagles (three systems with three alleles each could explain the results). These observations may be relevant to kidney transplantation in man, as further improvements in the effect of histocompatibility matching may be obtained by pursuing attempts to match for antigens of as yet undefined non-MHC rather than MHC systems.

Introduction

Matching donor and recipient for the serologically defined (SD) antigens of the major histocompatibility complex (MHC) drastically improves human kidney allograft survival in *related* donor-recipient combinations (1, 15, 20, 21). Matching for these SD antigens improves kidney allograft survival to a lesser extent in *unrelated* donor-recipient combinations (4, 5, 14, 17, 18, 21, 23). An explanation for the difference in efficacy of matching between related and unrelated donor-recipient combinations could be that the products of other genes within the MHC, different from the presently defined SD antigens, are more relevant for kidney allograft survival than the SD antigens themselves. An alternative explanation would be that the cumulative effect of non-MHC antigens, which is stronger in unrelated compared to related donor-recipient pairs, plays an important rôle in the determination of allograft survival. Finally, both hypotheses may be true and contribute each to the discrepancy in results of matching related compared to unrelated individuals.

A preclinical animal model such as the dog, in which an extensive immunogenetical knowledge has been gathered in the past (9, 24, 26), is optimally suited for a further evaluation of the effect of histocompatibility matching on allograft survival. Studies in such an experimental model have several advantages over human studies. Immunosuppressive regimes can be standardized and unintentional preimmunization for histocompatibility antigens can be more easily avoided. The selection of various genetically different donor-recipient pairs in dogs is relatively easy due to a somewhat limited heterogeneity in selected but still outbred breeds as the beagle (16) and a high standard of histocompatibility testing (9, 24, 26).

Previous studies in our laboratory demonstrated that matching for the SD antigens of the canine MHC, the DLA complex, drastically improves renal allograft survival in unimmunosuppressed related beagles (28). Preliminary results regarding the relative influence of subregions of the DLA complex to renal allograft survival in unrelated mongrels have been reported earlier (29). A more extensive study, including the results of kidney allograft survival in DLA recombinants is described elsewhere (chapter 6). In this report, the relative importance of non-MHC histocompatibility systems to renal allograft survival in unimmunized dogs is evaluated. Retrospectively, the possible rôle of sex-linked histocompatibility systems is investigated. A fairly large number of renal allografts were performed in unimmunosuppressed beagle littermates, making a reliable estimate of the number of minor histocompatibility systems and their alleles involved in the rejection of kidney allografts in this breed possible.

Material and methods

Dogs

Beagles were obtained from the colonies of the Centraal Proefdierenbedrijf TNO, Austerlitz, the Netherlands (Dr. J. van Vliet). Mongrels were obtained from local dog handlers and breeders. No predigrees were available for the mongrels. The chances of a close genetic relationship between these animals were decreased by appropriate instructions to the dog handlers, the collection of animals over a period of a year and the use of donors and recipients of different purchase dates. The pedigrees of the beagles were checked as far as possible (2-4 generations). No evidence for inbreeding was found. However, common ancestors were found in 9 out of 15 non-sibling donor-recipient combinations.

Serology and experimental groups

Serological testing was done with a battery of sixty to ninety alloantisera, using a one stage microlymphocytotoxicity test as described before (24). DLA matching was performed using different methods in the following experimental groups.

Group *1* were DLA identical littermate beagles in which no MLR was performed (n = 13). DLA haplotyping and matching was done by analyzing the segregation patterns of the typing sera in the families (24).

Group *2* were also DLA identical littermate beagles (n = 8). In this group SD matching was performed by means of segregation analysis and typing, i.e. by assigning SD specificities to the individuals. All pairs were MLR negative.

Group *3* were non-sibling beagles (n = 6). They were assumed to be DLA compatible on the basis of the reaction patterns of the battery of antisera with the lymphocytes of the donor and recipient. Arbitrarily, dogs were called SD identical when less than five sera reacted differently (positively or negatively) in the microlymphocytotoxicity test with donor and recipient lymphocytes.

Group *4* were SD and MLR identical non-sibling beagles (n = 9). SD matching was performed by means of SD typing.

Group *5* were SD and MLR identical unrelated mongrels (n = 5). SD compatibility was assessed by means of SD typing.

Group *6* were SD and MLR different unrelated mongrels (n = 8). All were SD typed.

Currently the SD antigens of the DLA complex are thought to be controlled by three loci. The antigens 1, 2, 3, 7, 8, 9 and 10 belong to the first or DLA-A series; the antigens 4, 5, 6, 13 and R 20 belong to the second or DLA-B series

and the antigens 11, 12 and R 15 belong to the third or DLA-C series. All series still have blanks and additional preliminary recognized antigens. Antigens number 1 to 13 have been confirmed by other workers and have been recommended for international use by the IUIS subcommittee for dog histocompatibility structures (Joint Report of the Second International Workshop on Canine Immunogenetics, 9).

Mixed lymphocyte reaction (MLR)

Unilateral cultures were performed as described previously (3). Identity for the lymphocyte defined (LD) antigens is based on the absence of significant stimulation in MLR in the presence of positive controls. Part of the mixed lymphocyte reactions of the mongrels was performed by van den Tweel and co-workers in a slightly different technique (22).

The following homozygous LD typing cells were available and used for the selection of unrelated DLA identical beagles (group *4*): DLA-50, DLA-51, DLA-52, DLA-53, DLA-54, DLA-55, DLA-56, DLA-57, DLA-58, R1 and R2. DLA plus a number refer to the nomenclature recommendations of the Second International Workshop on Canine Immunogenetics (9); R plus a number refer to homozygous typing cells which are presently only locally available (Rotterdam) and await a more official designation after international comparison.

Kidney allografting

Kidneys were transplanted to the iliac fossa as described before (28). A simultaneous bilateral nephrectomy of the recipients own kidneys was performed. None of the recipients was immunized for histocompatibility antigens by pregnancies, transfusions or other means. As the end point of graft survival was considered the postoperative day on which the animal died from renal insufficiency or when the serum creatinine rose above 1000 μ mole/l. Histological confirmation of rejection was always obtained. Technical failures were excluded from the analysis.

Statistical analysis

Survival data were analyzed statistically by means of one sided (P_1) or doubly sided (P_d) Wilcoxon rank sum tests. Recipients surviving for more than 150 days were excluded for the calculation of mean survival times (m.s.t.'s) ± standard deviations.

Table 5.1. Prolonged kidney allograft survival in DLA identical siblings without immunosuppression in one laboratory

Experiment	Reference	N[a]	mst ± sd[b]	Number of survivors > 150 days
1	28,[c]	13	43,0 ± 12,5	1
2	[c]	8	43,7 ± 18,0	2
donor blood prior to grafting	12	8	25,0 ± 19,8	2
third party unrelated blood prior to grafting	[d]	11	30,6 ± 11,2	2
third party unrelated blood after grafting	2,[e]	10	34,4 ± 9,2	2
third party related blood prior to grafting	[f]	5	30,5 ± 13,1	1
placenta eluate prior to and after grafting	[g]	3	30,4 ± 10,1	0
total		58		10 = 17 %[h]

[a] number of experimental animals studied, technical failures excluded
[b] mean survival time ± standard deviation, long survivors excluded
[c] this report
[d] Bull et al., in preparation
[e] own unpublished data
[f] Obertop et al., in preparation
[g] Jeekel et al., unpublished data
[h] 95% confidence limits 10-29%

Immunogenetic calculations

According to the formulas of Simonson (19), modified from Newth (11), the proportion of sibling to sibling donor-recipient combinations, which may expected to be compatible for the histocompatibility systems is $(\frac{n^3 + n^2 + 11n-9}{4\ n^3})^L$; the proportion of unrelated donor-recipient pairs, which may be expected to be histocompatible is $(\frac{4n - 3}{n^3})^L$. In both formulas n is the number of alleles and L the number of allelic series. For simplicity equal strength and frequency of the alleles and an equal number of alleles per series is assumed. The application of these formulas to the fraction of compatible pairs found in transplantation exper-

Table 5.2. DLA-SD typing. MLR and renal allograft survival of the donor-recipient combinations of group *2* and *4*

Dog (sex)[a]		DLA			MLR			
Recipient	Donor	A	B	C	Test-[b] value	Autol.[b] control	Maximal[b] response	survival (days)
Group 2								
D 6465 (M)	D 6466 (M)	9	6,13?	12	5	4	405	19
D 6513 (M)	D 6512 (M)	2,9	5,6	11,12	1	2	401	31
5522 (F)	5521 (F)	9	4,6	12	4	5	117	42
6471 (F)	6472 (F)	2	5,13	11	2	3	397	46
6472 (F)	6471 (F)	2	5,13	11	2	2	214	53
D 6466 (M)	D 6465 (M)	9	6,13?	12	2	5	636	71
D 5715 (M)	D 5713 (M)	2,9	5,6	11,12	2	1	21	>150
D 6210 (M)	D 6208 (M)	7,8	5	11	1	1	148	>150
Group 4								
D 55 (M)	D 5701 (M)	2,9	5,6	11,12	4	4	193	16
691 (F)[c]	D 5712 (M)[c]	2,9	4,5	11,12	4	2	89	22
D 5701 (M)	D 5528 (M)	2,9	5,6	11,12	3	6	469	22
D 5703 (M)	D 5528 (M)	2,9	5,6	11,12	2	7	186	24
652 (F)	D 5477 (M)	2,9	4,5	11,12	3	4	257	29
689 (F)[c]	D 5712 (M)[c]	2,9	4,5	11,12	3	2	89	31
D 5714 (M)[c]	D 5689 (M)[c]	9	4	12	4	3	63	34
D 5712 (M)[c]	689 (F)[c]	2,9	4,5	11,12	3	4	290	36
D 5689 (M)[c]	D 5714 (M)[c]	9	4	12	5	4	168	36

[a] M = Male, F = Female
[b] Counts per minute x 10^{-2}
[c] A common ancestor was found in the last 2-4 generations of the pedigree

iments makes a rough estimate possibie of the involved number of histocompatibility systems and their alleles. For this estimate 58 kidneys, all transplanted to DLA identical unimmunosuppressed littermate beagles in our laboratory (table 1), were considered as well as 15 kidneys from non-sibling donors (group *3* and *4*).

Results

Renal allograft survival in littermate and non-sibling dogs

The results of DLA typing, MLR and graft survival of group *2* and *4* are presented in table 2. Group *5* and group *6* are only presented for comparison; the details of SD typing and MLR will be presented in chapter 5. Group *1* is published in more detail before (28). In the littermate donor-recipient combin-

ations of group *1*, DLA identity was only assessed by means of serology. The DLA identity of the comparable dogs of group *2* was established by means of serological typing as well as by means of mixed lymphocyte reactions. Due to the low recombination frequency between the SD and LD loci (0.7%) no discrepancy in graft survival is expected when kidneys are taken from DLA SD matched or DLA SD and LD matched identical littermate donors.

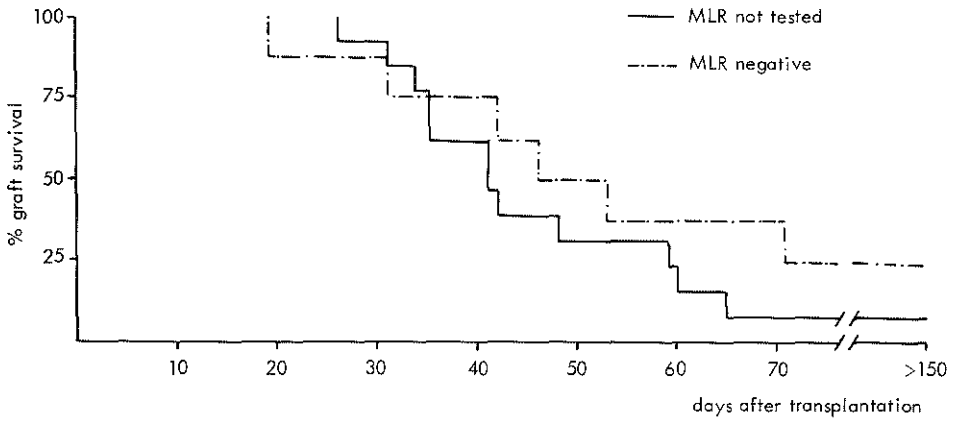

Figure 5.1. Kidney allograft survival in 21 DLA-SD identical littermate donor-recipient pairs. No significant difference is seen between MLR not tested (group 1) and MLR negative (group 2) combinations.

Figure 1 shows, that indeed no discrepancy is found ($P_1 = 0.22$). In one recipient a relative short survival time is seen of 19 days. However, the SD typing in that family was uncertain, thus an SD/LD recombination cannot be excluded with certainty.

In the non-littermate donor-recipient combinations of group *3*, DLA identity was only assessed by means of serology. The DLA identity of the comparable dogs of group *4* was established by means of both serology and mixed lymphocyte reactions. Due to the strong linkage disequilibrium between SD and LD genes in beagles (SD identical beagles are almost always LD identical -7; own unpublished observations) no discrepancy in the results of these two groups is expected either. Figure 2 shows that the results of group *3* and *4* are strikingly similar ($P_1 = 0.33$). Pedigree analysis (2-4 generations) of the donor-recipient combinations in group *3* and *4* demonstrated the presence of common ancestors in 9 out of the 15 combinations (4 out of 6 in group *3* and *5* out of 9 in group *4*. The mean survival time (m.s.t.) of the combinations in which common ancestors could be demonstrated was slightly longer than the m.s.t. in the combinations in which no common ancestors could be traced (m.s.t. 30.9 compared to 26.2 days), but the difference was not significant ($P_1 = 0.15$).

The survival times of group *4* are compared with the survival times of group

1 and *2* and with the survival times of kidney allografts from one and two haplotype different littermate donors, which were performed in the same laboratory (28) (figure 3). It appears that renal allograft survival in DLA LD and SD identical non-sibling donor-recipient pairs is significantly shorter than in DLA identical littermate donor-recipient pairs ($P_l < 0.0005$); about as long as in one DLA haplotype different littermate combinations ($P_d = 0.41$); and longer than in two DLA haplotype different littermate combinations ($P_d < 0.005$).

SD and LD matched unrelated (group *5*) mongrel to mongrel donor-recipient pairs have a shorter survival than non-sibling SD and LD matched beagles (group

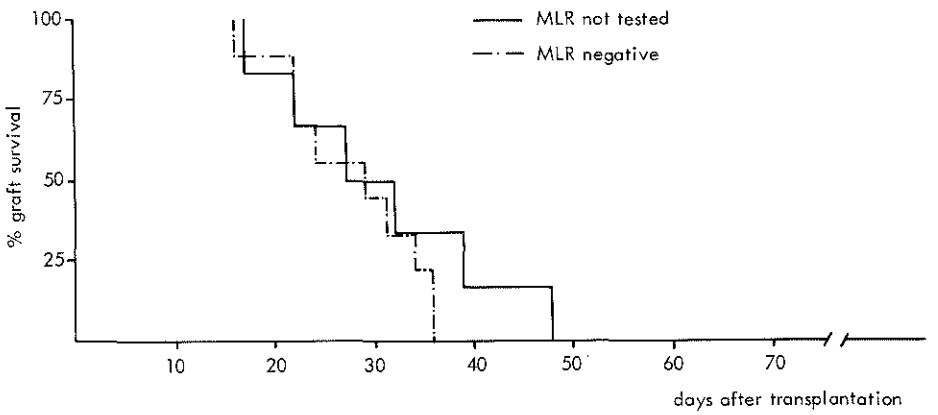

Figure 5.2. Kidney allograft survival in 15 DLA SD identical non-sibling donor-recipient pairs. No significant difference is seen between MLR not tested (group 3) and MLR negative (group 4) combinations.

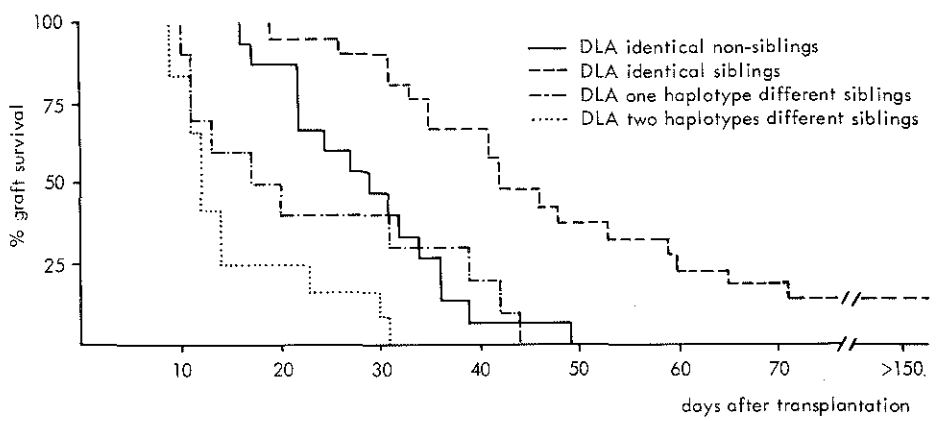

Figure 5.3. Kidney allograft survival in DLA matched non-sibling donor-recipient combinations and DLA identical, one haplotype different and two haplotypes different littermate donor-recipient combinations. The survival times in non-siblings are best comparable to those in one haplotype different donor-recipient combinations.

Table 5.3. Sex differences and renal allograft survival in unimmunized, not immunosuppressed, DLA matched littermate and non-sibling donor-recipient combinations

Experiment	sex[a] donor→recipient	N[b]	Survival times[c]
1 + 2	M → M	9	19,31,31,35,41,60,71,>150,>150
	M → F	3	35,41,59
	F → M	3	42,48,65
	F → F	6	26,33,42,46,53,>150
3 + 4	M → M	5	16,22,24,34,36
	M → F	5	17,22,27,29,31
	F → M	2	36,39
	F → F	3	22,32,48

[a] M = male F = Female
[b] number of experimental animals within each group
[c] median survival times bold

4), $P_1 < 0.02$. The m.s.t.'s of groups *1, 2, 3, 4* and *5* are all significantly longer than the m.s.t. of unrelated mismatched mongrels (P_1 for group *1, 2, 3* and *4* each < 0.0005, for group *5* < 0.02).

Sex-linked histocompatibility

In table 3, the data of group *1* and *2* are rearranged to permit a search for the possible presence of sex-linked histocompatibility antigens. Details of the methodology of such an analysis are described elswhere (25). Survival of male to female grafts was no shorter than survival of male to male grafts. This is in contrast with the expected shorter survival of male to female grafts in the presence of important Y-linked histocompatibility systems. Furthermore, survival of the female to male grafts was no shorter than survival of male to male and female to female grafts, and the distribution in these groups seems to be similar. This argues against an important influence of X-linked histocompatibility genes in this model.

The number of minor histocompatibility systems and their alleles

It appears from table 1, that 17% (95% confidence limits 10-29%) of the kidneys, transplanted to DLA identical littermates, survived longer than 150 days. This percentage presents the maximum number of pairs compatible for non-MHC antigens. Figure 4[a] shows that a limited number of minor histocompatibility sys-

tems with a small number of alleles (for instance two systems with four alleles, three systems with three alleles, or four systems with two to three alleles each) may explain the results. It can be calculated (see also figure 4[b]), that, based on this assumption, about 4% of compatible pairs are expected to be found in random unrelated donor-recipient combinations. This fits with the observation that no prolonged survival times were found in non-sibling DLA identical beagle donor-recipient pairs, i.e. 0% (95% confidence limits 0-20%) compatible pairs.

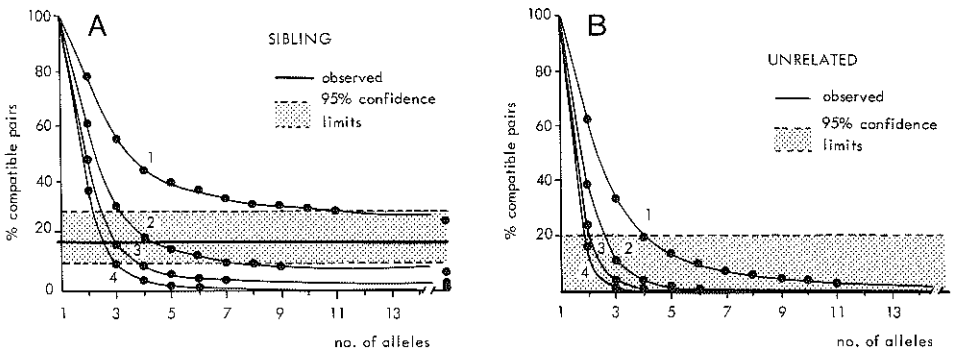

Figure 5.4. Estimation of the number of minor histocompatibility systems and their alleles involved in the determination of renal allograft rejection. The found % of rejected kidneys with the 95% confidence limits are indicated in the figure. The curves represent the expected % of rejected kidneys as a function of the number of the alleles, assuming equal strength and frequency, for 1, 2, 3 and 4 minor histocompatibility systems
a. for renal allograft survival in 58 unimmunosuppressed DLA identical beagle littermate donor-recipient combinations.
b. for renal allograft survival in 15 unimmunosuppressed DLA identical beagle non-sibling donor-recipient combinations.

Discussion

A considerable amount of knowledge exists on minor histocompatibility systems in mice. In that species, the cumulative effect of minor histocompatibility differences can equal the influence of MHC differences with regard to skin graft survival (6). However, little is known of the role of minor histocompatibility systems to allograft survival in other experimental models. Data in this area are needed for an estimate of the maximum benefit that can be obtained from matching for antigens of the MHC for renal allograft survival in unrelated human donor-recipient pairs.

The analysis of the results of kidney allografting experiments in a preclinical model such as the dog may provide a prognosis on the effect of matching for

more or different antigens of the MHC other than the usual SD antigens HLA-A, HLA-B and HLA-C. This study shows that highly reproducible results are obtained in littermate and non-littermate donor-recipient pairs.

Furthermore, it is of interest to note that the similarity of this model with the results of small bowel grafting are striking (27). In that model the mean survival times were 7.6 days for unrelated mongrels, 27.5 days for non-sibling matched beagles and 45.3 days for matched littermate beagles. This indicates that the antigens involved in the rejection of small bowel and renal allografts are the same.

Renal allografts have been performed in non-sibling *beagles*, because identity for the LD and for SD antigens in this race probably means identity for all antigens of the DLA complex. This is suggested by the extremely strong linkage disequilibrium between DLA-A, -B, -C and -D genes (7, 9, 22, 26 and own unpublished data). This linkage disequilibrium is probably caused by a pronounced founder effect (26). Furthermore, common ancestors could be identified in most of the donor-recipient pairs, and might have been traced in the other donor-recipient combinations if a more extensive pedigree analysis could have been performed.

The availability of non-sibling MHC identical beagles offers an excellent possibility for the determination of the relative importance of non-DLA versus DLA histocompatibility. Differences in non-DLA (minor histocompatibility) antigens will be less frequent in littermate compared to non-littermate donor-recipient combinations. If indefinite numbers of alleles for all systems are assumed, littermates will have 50% of their genome in common, while unrelateds will have nothing in common. In such a situation the number of minor histocompatibility differences in unrelated combinations will be twice the number of histocompatibility differences present in littermate combinations. In our experimental situation, this would mean that the cumulative strength of non-DLA histocompatibility structures is comparable to that of the DLA histocompatibility structures, as the graft survival in the one haplotype different littermate group (m.s.t. 23.8 ± 13.8 days) is comparable to the graft survival in the DLA matched unrelated group (m.s.t. 29.0 ± 8.8 days). As the number of alleles of the histocompatibility systems is found to be limited, the discrepancy between the littermate and non-littermate groups will be less; also additional accidental matches for non-DLA histocompatibility systems may be present in the non-sibling group because donors and recipients are related. Both are reasons to assume that our estimate that the cumulative effect of non-DLA histocompatibility systems is comparable to the strength of the DLA histocompatibility systems is an underestimate. This is illustrated by the observation that the survival times in SD and LD identical mongrels, which also may be identical for all antigens of the DLA complex, are still much shorter (m.s.t. 18.2 ± 4.6 days)

than in non-sibling beagles. A possible explanation for this finding is a greater heterogeneity of non-DLA histocompatibility systems in mongrels. It is possible that part of these non-DLA histocompatibility systems are loosely linked to the histocompatibility systems of the DLA complex, but proof for such a linkage is not available.

The possibility that outside the DLA complex only a limited number of minor loci (for instance three autosomal loci with three alleles each) would control kidney allograft survival is rather unexpected in view of the many loci influencing mouse skin graft survival. However, it should be realized that this is a (maybe slight) underestimate as

a) the beagles are a selected breed with a somewhat limited heterogeneity
b) incompatibilities for minor histocompatibility systems may occur in spite of prolonged kidney allograft survival and
c) the involved alleles are unlikely to be of equal frequency and strength.

On the other hand, the last possibility in particular makes attempts to match for minor histocompatibility antigens attractive. If two or three alleles were much stronger and more frequent than others, matching for their respective antigens might effect a prolongation of graft survival in a considerable number of recipients. The logistics of matching for these antigens would however not be easy. Previous experience has shown that it is difficult to produce serological reagents recognizing minor histocompatibility antigens. However, the described observations call for all sera which are (10) or become available to be tested for their relevance to allograft survival in a controlled model.

It has been shown that sex-linked genes may be of some importance in determining graft survival in unimmunized recipients (25). The data described here however do not show an important influence of sex-linked genes in the unimmunized recipient. More extensive data are awaited before a decision can be taken on the usefulness of matching for sex for the selection of donor-recipient pairs for further experimental studies.

In experimental animal studies, isolated weak histocompatibility differences appear to be easier to suppress with immunosuppressive drugs than strong histocompatibility differences. In humans, comparable data are lacking. Furthermore, there is no evidence that differences for weak histocompatibility systems can easily be suppressed if their cumulative effect is strong (8). In fact, data in human kidney allografting suggest that minor histocompatibility structures in related donor-recipient combinations are as strong as a one haplotype difference (15). Furthermore, it appeared that on the American continent HLA matching is not effective in centers with a good overall outcome of clinical cadaver transplantation (13). This might indicate that with the optimal use of immunosuppression, differences for MHC antigens are as equally well suppressed as non-MHC differences.

Observations in the beagle model indicate that the efficacy of current human selection procedures might be increased when more emphasis is placed on the development of selection procedures for minor histocompatibility systems. Incompatibility for such systems would be only of clinical relevance of they withstood regular, currently available immunosuppressive therapy. A proper evaluation of this problem requires the use of an experimental animal model. Therefore, investigations to study the importance of different immunogenetic situations for renal allograft survival under immunosuppression in dogs are currently underway.

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Literature cited

1. BRYNGER H, SANDBERG L, AHLMEN H, et al: 1977 Transplant Proc 9:479
2. BIJNEN A B, VRIESENDORP H M, DE GRUYL J, et al: 1975 Transplant Proc 7:431
3. BIJNEN A B, VRIESENDORP H M, GROSSE-WILDE, et al: 1977 Tissue Antigens 9:187
4. DAUSSET J, HORS J, BUSSON M et al: 1974 New Engl J Med 290:979
5. FESTENSTEIN H, SACHS J A, PARIS A M I, et al: 1976 Lancet i : 157
6. GRAFF R J, SILVERS W K, BILLINGHAM R E, et al: 1966 Transplantation 4:605
7. GROSSE-WILDE H, VRIESENDORP H M, NETZEL B et al: 1975 Transplant Proc 7:159
8. HILDEMANN W H: 1970 Transplant Rev 3:5
9. JOINT REPORT OF SECOND INTERNATIONAL WORKSHOP ON CANINE IMMUNOGENETICS: 1976 Transplant Proc 8:289
10. KRUMBACHER K, SCHNAPPAUF H: 1976 Tissue Antigens 7:181
11. NEWTH D R: 1961 Transplant Bull 27:452
12. OBERTOP H, JEEKEL J, VRIESENDORP H M, et al: 1975 Transplantation 20:49
13. OPELZ G, MICKEY M R, TERASAKI P I: 1977 Transplantation 23:490
14. OPELZ G, MICKEY M R, TERASAKI P I: 1977 Transplant Proc 9:27
15. OPELZ G, TERASAKI P I: 1977 Transplantation 24:106
16. REHFELD C E: 1970 Am J Vet Res 31:723
17. SACHS J A, FESTENSTEIN H, TUFFNELL V A et al: 1977 Transplant Proc 9:483
18. SCANDIATRANSPLANT REPORT: 1975 Lancet i : 240
19. SIMONSON M: 1966 Transplantation 4:354
20. SINGAL D P, MICKEY M R, TERASAKI P I: 1969 Transplantation 7:246
21. SOLHEIM B G, FLATMARK A, ENGER E, et al: 1977 Transplant Proc 9:475
22. VAN DEN TWEEL J G, VRIESENDORP H M, TERMIJTELEN A, et al: 1974 J Exp Med 140:825
23. VAN HOOFF J P, VAN DER STEEN G J, SCHIPPERS H M A, et al: 1972 Lancet ii : 1385
24. VRIESENDORP H M: 1973 Thesis, Erasmus University Rotterdam, Bronder Offset, Rotterdam, the Netherlands
25. VRIESENDORP H M, BIJNEN A B, VAN KESSEL A C M, et al: 1978 Exp Haemat Today, Vol. II, Springer, New York, in press
26. VRIESENDORP H M, GROSSE-WILDE H, DORF M E: 1977, p. 129. In: Götze D (ed), The major histocompatibility system in man and animals, Springer, Heidelberg, Germany
27. WESTBROEK D L, ROTHENGATTER C, VRIESENDORP H M, et al: 1970 Europ Surg Res 2:401
28. WESTBROEK D L, SILBERBUSCH J, VRIESENDORP H M, et al: 1972 Transplantation 14:582
29. WESTBROEK D L, VRIESENDORP H M, VAN DEN TWEEL J G, et al: 1975 Transplant Proc 7:427

Chapter 6 has been submitted for publication

CHAPTER 6

GENETICS OF KIDNEY ALLOGRAFT SURVIVAL IN DOGS

II Relevance of subregions of the major histocompatibility complex in recipients without immunosuppressive therapy

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Summary

To assess the relative importance of the subregions of the canine major histocompatibility complex (MHC) for renal allograft survival in recipients without immunosuppressive therapy, kidneys were transplanted in two experimental groups. The results in six beagle donor-recipient pairs in which the donor or recipient had a recombination in the MHC, fit with a predominant role of the subregion which contains the major mixed lymphocyte reaction (MLR) locus. Results in unrelated mongrels also show that compatibility for MLR is more favourable for kidney allograft survival than compatibility for the serologically defined (SD) antigens. However, the effect of combined matching for MLR and SD antigens in unrelated dogs is slight in comparison to the effect of MLR and/or SD matching in related dogs. This indicates that other important histocompatibility systems must exist in this species.

Introduction

Matching for the serologically defined (SD) antigens of the major histocompatibility complex (MHC) in sibling donor-recipient combinations drastically improves human kidney allograft survival (3, 12, 15, 16). Matching for the SD antigens in unrelated donor-recipient combinations only slightly improves renal allograft prognosis (6, 8, 10, 13, 14, 16, 19). Thus, some parts of the MHC may be more important to allograft survival than others.

To study the relative importance of the various subregions of the major histocompatibility complex (MHC), two methods are available. In the first place, animals with a recombination in the MHC can be used for renal allografting experiments in related donor-recipient combinations. The survival times are indicative for the histocompatibility effect of the subregions on both sides of the crossing-over site. The scarce availability of recombinants over long periods is a severely limiting factor to the performance of such studies in outbred species. Secondly unrelated donor-recipient pairs can be used which are identical for some but different for other defined markers of the MHC. The results of such studies have to be interpreted with care as matching for defined markers in the MHC may entail unintentional matching for undefined antigens, when linkage disequilibrium exists between alleles of the defined and undefined genes of the MHC.

The dog model has been developed over the last years as an animal model which is optimally suited to study the effect of histocompatibility matching on allograft survival (9, 20, 21, 22, 23).

Preliminary results regarding the relative influence of matching for the products of the subregions of the DLA complex to renal allograft survival have been reported earlier (24). In this report a more extensive study including the results of kidney graft survival in DLA recombinants is presented, with special regard to the relevance of compatibility for the MLR or lymphocyte defined (LD) antigens.

Material and methods

Dogs

Beagles were obtained from the colonies of Het Centraal Proefdierenbedrijf TNO, Austerlitz, the Netherlands (Dr. J. van Vliet). Mongrels were obtained from local dog handlers and breeders. No pedigrees were available for the mongrels. The chances of close genetic relationships between these animals were decreased by appropriate instructions to the dog handlers, the collection of animals over the period of a year, and the use of donors and recipients from different purchase dates.

Serology

A battery of seventy to ninety different alloantisera was used in a one stage microlymphocytotoxicity test to type for the SD antigens as described before (20, 22). Currently the SD antigens of the DLA complex are thought to be controlled by three loci. The antigens 1, 2, 3, 7, 8, 9 and 10 belong to the first or DLA-A series; the antigens 4, 5, 6, 13 and R 20 belong to the second or DLA-B series and the antigens 11, 12 and R 15 belong to the DLA-C series. All series still have blanks and additional preliminary recognized antigens. Antigen number 1 to 13 have been confirmed by other workers and have been recommended for international use by the IUIS subcommittee for dog histocompatibility structures (Joint Report of the Second International Workshop on Canine Immunogenetics, 9).

Mixed lymphocyte reaction

Unilateral cultures were performed as described previously (4). Part of the mixed lymphocyte reactions of the mongrels were performed in a slightly different technique by van den Tweel and co-workers (17). The unrelated mongrels were presumed to be LD matched when the reactivity in MLR of the recipients lymphocytes as responder cells against the donors lymphocytes as stimulator cells was less than 20% of the maximal response in the same test. The following homozygous LD typing cells were available and used during part of this study: DLA-50, DLA-51, DLA-52, DLA-53, DLA-54, DLA-55, DLA-56, DLA-57, DLA-58, R1 and R2. DLA plus a number refer to the nomenclature recommendations of the Second International Workshop on Canine Immunogenetics (9); R plus a number refer to homozygous typing cells which are presently only locally available (Rotterdam) and await a more official designation after international comparison.

GT

The determination and the genetics of the immune response against copolymers of L-glutamic acid with L-tyrosine (GT) in dogs is described elsewhere (22). In brief, the IrGT region was found to be linked to the DLA complex. The most probable chromosomal sequence is IrGT : DLA-D : DLA-A : DLA-B.

Kidney allografting

Kidneys were transplanted to the iliac fossa as described before (23). A simultaneous bilateral nephrectomy of the recipients own kidneys was performed. The

recipients were not immunized and received no immunosuppression or supportive therapy, except an SD/LD recombinant, which received inadvertently three blood transfusions from unrelated animals as part of another experimental protocol. This is not considered relevant for the interpretation of the results as transfusions in this protocol did not prolong renal allograft survival (Bull et al., in preparation). The postoperative day on which the animals died from renal failure or when the serum creatinine rose above 1000 μ Mole/l, caused by immunological rejection of the graft, was taken as the end point of graft survival. Histological confirmation of rejection was always obtained.

Kidney allografting was performed in the following experimental groups:

a) Related, DLA recombinant beagles.

Three dogs with a recombination in the MHC could be used for six donor-recipient pairs: The kidney of an animal with a recombination between DLA-A and DLA-B was transplanted to a littermate which differed for one DLA-B antigen alone. The second kidney of the same recombinant was exchanged with the kidney of another sib which was identical to the first recipient. The MLR between the recombinant and the other two recipients was positive in both directions. The SD/LD recombinant donated its kidney to an SD different LD identical sibling. A dog with a recombination between the MHC and the immune response locus for GT (22), who was a "GT responder", exchanged kidneys with a DLA SD and LD identical littermate, who was a "GT non-responder".

b) Unrelated mongrels.

Four different groups were grafted:

Group *1*: SD and LD identicals (n = 5);

Group *2*: SD different and LD matched dogs (n = 5);

Group *3*: SD identical LD different dogs (n = 11);

Group *4*: SD different LD different dogs (n = 8).

Statistical evaluation of the survival times was performed by means of one sided Wilcoxon rank sum tests.

Results

Recombinants

Table 1 shows the result of the MLR of an individual with a recombination between the DLA-A and -B loci with its parents, sibs and relevant typing cells. The test has been repeated twice with the same outcome. It is remarkable that the recombinant shows a low (but definite) stimulation with both its "three quarter" DLA identical sibs and both typing cells of the father. As the recombinant

Table 6.1. MLR of dog D5.687 with a paternal recombination between DLA-A and several members of its family

Dog	SD genotyping	D2.175$_x$	2.292$_x$	D5.687$_x$	D5.690$_x$	D5.691$_x$[e]	D5.688$_x$	D5.689$_x$	Unrelated$_x$	58 hz$_x$	R2 hz$_x$[a]
D2.175 (father)	A9-B4-C12/A7-B–-C—	1 (± 33%)[b]	23 (± 10%)	16 (± 13%)	19 (± 10%)	NT[c]	1 (± 24%)	1 (± 56%)	49 (± 7%)	1 (± 35%)	1 (± 28%)
2.292 (mother)	A9-B4-C12/A3-B–-C—	10 (± 16%)	1 (± 72%)	5 (± 23%)	1 (± 38%)	NT	1 (± 35%)	1 (± 51%)	15 (± 12%)	1 (± 38%)	3 (± 16%)
D5.687[d] (recombinant)	A9-B—C—/A3-B–-C—	33 (± 5%)	9 (± 28%)	0 (± 14%)	5 (± 49%)	40 (± 32%)	10 (± 16%)	7 (± 19%)	12 (± 33%)	8 (± 27%)	5 (± 20%)
D5.690	A9-B4-C12/A3-B–-C—	13 (± 11%)	0 (± 60%)	5 (± 14%)	0 (± 37%)	0 (± 10%)	1 (± 9%)	0 (± 38%)	16 (± 7%)	1 (± 35%)	3 (± 16%)
D5.691[e]	A9-B4-C12/A3-B–-C—	NT	NT	38 (± 17%)	0 (± 21%)	0 (± 10%)	NT	NT	NT	NT	NT
D5.688	A9-B4-C12/A9-B4-C12	13 (± 14%)	9 (± 38%)	13 (± 15%)	6 (± 28%)	NT	0 (± 40%)	0 (± 35%)	22 (± 2%)	0 (± 70%)	3 (± 66%)
D5.689	A9-B4-C12/A9-B4-C12	11 (± 8%)	6 (± 41%)	12 (± 9%)	5 (± 18%)	NT	0 (± 70%)	1 (± 16%)	25 (± 14%)	0 (± 56%)	1 (± 39%)
Unrelated	A9-B6-C12/A2-B5-C11	42 (± 9%)	12 (± 7%)	8 (± 26%)	11 (± 14%)	NT	8 (± 23%)	6 (± 19%)	1 (± 56%)	8 (± 28%)	4 (± 6%)

[a] 58 hz and R2 hz: homozygous typing cells specific for the father
[b] cpm x 10^{-3} (± standard deviation %)
[c] NT: not tested
[d] The positive reaction with both paternal typing cells (58 hz and R2 hz) can be explained by the assumption of two loci for MLR
[e] Test results of D5.691 were obtained in a separate test.

Table 6.2. MLR of SD/LD recombinant 4.341 with four sibs

Dog.	SD genotyping	4.341$_x$	D4.339$_x$	D4.335$_x$	D4.342$_x$	D4.337$_x$
4.341	A9-B4-C12/A2-B5 -C11	3[a] (± 9%)	3 (± 24%)	17 (± 32%)	17 (± 32%)	40 (± 26%)
D 4.339	A9-B4-C12/A9-B4 -C12	2 (± 74%)	5 (± 58%)	17 (± 42%)	12 (± 30%)	53 (± 35%)
D 4.335	A9-B4-C12/A2-B5 -C11	3 (± 11%)	4 (± 31%)	3 (± 15%)	13 (± 19%)	21 (± 34%)
D 4.342	A9-B4-C12/A1-B13-C—	3 (± 2%)	5 (± 53%)	13 (± 41%)	3 (± 47%)	33 (± 62%)
D 4.337	A2-B5-C11/A1-B13-C—	20 (± 31%)	25 (± 23%)	28 (± 60%)	15 (± 38%)	4 (± 32%)

[a] cpm x 10^{-2} (± standard deviation %)

Table 6.3. SD typing, GT response and MLR of DLA-D/IrGT recombinant 2.207

Dog	GT response		2.207_x	2.208_x	$DR013_x$
2.207	-	A1-B- -C—/A7-B4-C—	2[a] (± 60%)	2 (± 38%)	95 (± 9%)
2.208	+	A1-B- -C—/A7-B4-C—	2 (± 65%)	1 (± 36%)	75 (± 11%)
DR013	NT		66 (± 19%)	46 (± 20%)	3 (± 37%)

[a] cpm x 10^{-3} (± standard deviation %).

is not immunized, the outcome of the test can only be explained by assuming at least two loci for MLR.

Table 2 shows the MLR results of the SD/LD recombinant. Dog 4.341 is SD different from its homozygous sib D 4.339, but is identical in MLR; he is also SD identical with its sib D 4.335 but different in MLR. Thus, the kidney grafted from 4.341 to D 4.339 was SD different but LD identical.

Table 3 shows that the GT recombinant 2.207, which was a "GT non-responder", was SD and LD identical with its littermate 2.208, which was a "GT responder".

Table 6.4. Kidney allograft survival in DLA recombinant donor - recipient combinations

Recipient	Donor	Recombinant site	Survival time (days)
D5.691	D5.687	DLA - A/B	10
D5.690	D5.687	DLA - A/B	11
D5.687	D5.691	DLA - A/B	11[a]
2.207	2.208	DLA - D/ IrGT	19
2.208	2.207	DLA - D/ IrGT	31
4.342	4.341	LD/SD	>300

[a] dog D5.687 died from a volvulus with a normal serum creatinine but clear histological signs of rejection

The survival times of the kidney allografting experiments of these recombinants are given in table 4. Dog D 5.687 died on the 11^{th} postoperative day from a volvulus with a normal serum creatinine. However at histological examination of the graft severe signs of rejection were seen. Therefore it is reasonable to assume that otherwise he would have died of uremia in about another week. The survival times of the recombinant donor-recipient combinations are significant shorter than those of the DLA identical littermate donor-recipient combinations (see figure 1), which are described in greater detail elsewhere (23; chapter 5) ($p = 0.01$ including D 5.687 as 18 days; $p = 0.03$ excluding D 5.687). The MLR compatible recipients live longer than the MLR different recipients.

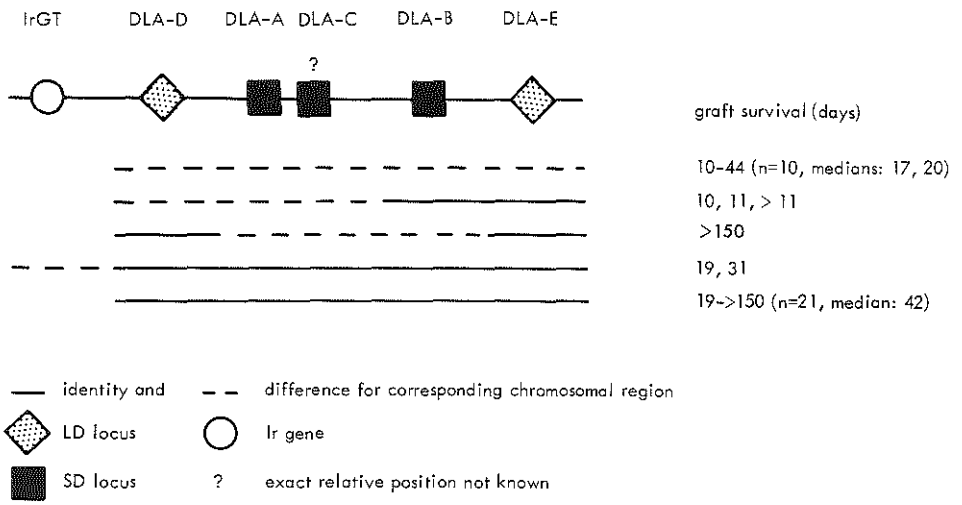

Figure 6.1. Genetic structure of the major histocompatibility complex in the dog and kidney allograft survival in six "three quarter" identical littermate donor-recipient pairs compared with the control groups of full-house DLA identical sibs and one DLA haplotype different sibs.

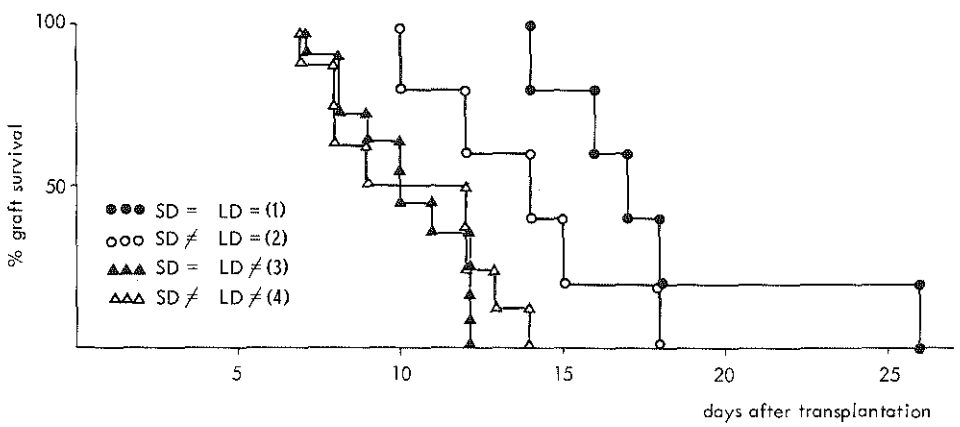

Figure 6.2. Kidney allograft survival in unrelated mongrel dogs in different donor-recipient combinations.

Unrelated mongrels

Table 5 gives the typing results of the four different groups of unrelated donor-recipient pairs. The survival times are illustrated in figure 2. The LD matched individuals survive significantly longer than the LD mismatched individuals (group *1* versus group *3*, $p = 0.001$; group *2* versus group *4*, $p = 0.04$; group *1* + *2* versus group *3* + *4*, $p < 0.001$). SD matching alone does not improve graft

Table 6.5. SD typing, MLR results and kidney allograft survival times in SD = LD = (group *1*), SD ≠ LD = (group *2*), SD = LD ≠ (group *3*), and SD ≠ LD ≠ (group *4*) unrelated donor-recipient pairs

Dog (sex)[a]		SD typing						MLR			
		Recipient DLA			Donor DLA						
Recipient	Donor	A	B	C	A	B	C	Test[b] value	Autol.[b] control	Maximal[b] response	Survival days
Group 1											
1398 (M)	04 (F)	3, 9	4	—	3, 9	4	—	6	2	588[c]	14
1308 (M)	R219 (F)	3, 9	6	—	3, 9	6	—	1	0	785[c]	16
033 (F)	021 (F)	3	5	12	3	5	12	2	1	641[c]	17
021 (F)	033 (F)	3	5	12	3	5	12	6	4	416[c]	18
1400 (M)	1342 (M)	3	5,10	—	3	5,10	—	13	16	748[c]	26
Group 2											
D705 (M)	0144 (F)	1, 2	5,13	11	2,10	5	11	14	3	71	10
073 (M)	1358 (F)	3, 9	4	—	3, 7	4	11	18	8	885[c]	12
1358 (F)	073 (M)	3, 7	4	11	3, 9	4	—	4	2	587[c]	14
1401 (M)	1338 (F)	3	5,13	11	3, 9	5,13	—	7	4	860[c]	15
0144 (F)	D705 (M)	2,10	5	11	1, 2	5,13	11	12	2	67	18
Group 3											
036 (M)	1364 (M)	7,10	5,13	—	7,10	5,13	—	1589	3	1589[c]	7
1364 (M)	036 (M)	7,10	5,13	—	7,10	5,13	—	1497	2	1497[c]	8
1407 (M)	1392 (F)	9	4, 5	—	9	4, 5	—	1125	3	1668[c]	8
1392 (F)	1407 (M)	9	4, 5	—	9	4, 5	—	873	2	873	9
1255 (M)	012 (M)	3, 7	5,13	—	3, 7	5,13	—	461	1	872[c]	10
023 (F)	1357 (F)	3, 7	5	12	3, 7	5	12	732	21	1527[c]	10
0145 (M)	5455 (F)	2, 9	4, 5	11,12	2, 9	4, 5	11,12	167	3	167	11
5455 (F)	0145 (M)	2, 9	4, 5	11,12	2, 9	4, 5	11,12	137	6	209	12
5456 (F)	0145 (M)	2, 9	4, 5	11,12	2, 9	4, 5	11,12	133	7	277	12
D704 (M)	0142 (F)	2, 9	4, 5	11,12	2, 9	4, 5	11,12	149	4	149	12
D706 (M)	0142 (F)	2, 9	4, 5	11,12	2, 9	4, 5	11,12	167	9	248	12
Group 4											
DR222 (M)	R220 (F)	3, 9	6	12	2, 3	5	11	330	1	631[c]	7
5478 (F)	0143 (M)	9	4, 6	11,12	10	6	—	78	2	179	8
R220 (F)	DR222 (M)	2, 3	5	11	3, 9	6	12	135	1	522[c]	8
1345 (M)	R220 (F)	3	–	11,12	2, 3	5	11	695	1	695[c]	9
D707 (M)	0141 (M)	9	4, 6	11,12	10	6	—	45	2	65	12
0141 (M)	D707 (M)	10	6	—	9	4, 6	11,12	18	2	26	12
688 (F)	0141 (M)	9	4, 6	11,12	10	6	—	61	2	80	13
0143 (M)	D5209 (M)	10	6	—	9	4, 6	—	72	3	112	14

[a] M = Male, F = Female

[b] counts per minute x 10^{-2}

[c] MLR performed by van den Tweel and co-workers

survival significantly: group *3* versus group *4*, p = 0.39; group *1* + *3* versus group *2* + *4*, p = 0.45, but a slight improvement of graft survival is seen in LD matched combinations (group *1* versus group *2*, p = 0.07).

Discussion

Matching for LD rather than for SD antigens of the MHC may improve human kidney allograft survival according to some authors (5, 8, 11). These data are supported by the results of experimental kidney allografting in dogs (1, 24) and rhesus monkeys (13), but not in rats (7) or pigs (2).

The results of the kidney allografting experiments in the recombinants reported in this study support the hypothesis that the subregion of the MHC, in which the locus is found that codes for the DLA-D antigens, plays a predominant role in the determination of renal allograft survival. This can be clarified on behalf of the current concept of the DLA complex (figure 1). The survival times of these kidneys allografts are expected to be in the range of DLA identical sibs or DLA one haplotype different siblings. The survival times of D 5.691 and D 5.690 (and D 5.687) only fit in the DLA one haplotype different group. This means that an important histocompatibility gene is located to the left of DLA-B on the figure. The prolonged survival of B 4.342 only fits in the DLA identical group. This means, that important histocompatibility structures are located to the left of DLA-A on the figure. The intermediate short survival times of B 2.207 and B 2.208 fit in both DLA identical and DLA one haplotype different groups. No GT studies were performed in these control groups; therefore recombinations between DLA-D and IrGT cannot be excluded. Thus, it is possible that important histocompatibility genes are located left to DLA-D on the figure, and that the shortest survivors in the DLA identical group had such a recombination between DLA-D and IrGT too. Thus, if one would prefer to adhere to the hypothesis, that only one gene or subregion of the MHC has a predominant role in the determination of renal allograft prognosis, than such a gene (or subregion) should be located outside DLA-A–B–C–D, close to DLA-D. Of course, on the basis of these limited data, the presence of more loci with an important role for renal allograft survival within the same region cannot be excluded.

The data in unrelated mongrels show that matching for MLR improves graft survival significantly, while matching for SD antigens alone does not. A slight beneficial effect of SD matching may be seen in MLR compatible donor-recipient combinations (compare group *1* with group *2*). However the difference between these groups may also be caused by slightly higher MLR test values in group *2* compared to group *1*. The effect of combined SD and LD matching in unrelated mongrels (median survival time = 17 days) is disappointing when compared with the effect of DLA matching in littermate donor-recipient pairs (median survival time = 42 days) (23; chapter 5).

Several explanations for the discrepancy between unrelated and related matched groups are available. One hypothesis is, that not the DLA-D locus itself but another locus (or loci) of the MHC is important for graft survival. If such

"major" graft prognosis loci were in linkage disequilibrium with the genes controlling MLR, identity for DLA-D would often result in partial identity for the "major" graft prognosis antigens. Complete identity however would be more rare. In this way LD matching would contribute to a slight improvement of graft prognosis. Another possibility is that the LD loci have a histocompatibility effect per se with respect to renal allograft survival and that the discrepancy with the DLA identical littermate group is caused by a greater degree of incompatibility for minor histocompatibility antigens in the unrelated donor-recipient pairs. These different hypotheses are discussed in more detail in chapter 5.

The renal allografting experiments described in this report are performed without the use of immunosuppression. It is possible that with the use of these drugs the histocompatibility effect of some antigens will become more pronounced than without these drugs. Therefore, histocompatibility studies with the use of immunosuppressive drugs are needed for a more extended evaluation of the relevance of matching for subregions of the MHC to kidney allograft survival.

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Literature cited

1. BACH ML, BACH FH, RASMUS W, et al: 1975 Transplant Proc 7 : 423
2. BRADLEY BA, WHITE DJG, CALNE RY, et al: 1973, p. 85, In: Lindahl – Kiessling K and Osoba D, (eds): Proceedings of Eight Leucocyte Culture Conference. Academic Press, New York
3. BRYNGER H, SANDBERG L, AHLMEN J, et al: 1977. Transplant Proc 9 : 479
4. BIJNEN AB, VRIESENDORP HM, GROSSE-WILDE H, et al: 1977 Tissue Antigens 9 : 187
5. COCHRUM KC, PERKINS HA, PAYNE RO, et al: 1973 Transplant Proc 5 : 391
6. DAUSSET J, HORS J, BUSSON M, et al: 1974 New Engl J Medic 290 : 979
7. FABRE JW, MORRIS PJ: 1974 Transplantation 18 : 429
8. FESTENSTEIN H, SACHS JA, PARIS AMI, et al: 1976 Lancet i : 157
9. JOINT REPORT OF THE SECOND INTERNATIONAL WORKSHOP ON CANINE IMMUNOGENETICS: 1976 Transplant Proc 8 : 289
10. OPELZ G, MICKEY MR, TERASAKI PI: 1977 Transplant Proc 9 : 27
11. OPELZ G, TERASAKI PI: 1977 Transplantation 23 : 375
12. OPELZ G, TERASAKI PI: 1977 Transplantation 24 : 106
13. SACHS JA, FESTENSTEIN H, TUFFNEL VA, et al: 1977 Transplant Proc 9 : 483
14. SCANDIATRANSPLANT REPORT: 1975 Lancet i : 240
15. SINGAL DP, MICKEY MR, TERASAKI PI: 1969 Transplantation 7 : 246
16. SOLHEIM BG, FLATMARK A, ENGER, E et al: 1977 Transplant Proc 9 : 475
17. VAN DEN TWEEL JG, VRIESENDORP HM, TERMIJTELEN A, et al: 1974 J Exp Med 140 : 825
18. VAN ES AA, MARQUET RL, VAN VREESWIJK W, et al: 1977 Transplant Proc 9 : 257
19. VAN HOOFF JP, VAN DER STEEN GJ, SCHIPPERS HMA, et al: 1972 Lancet ii : 1385
20. VRIESENDORP HM: 1973 Thesis, Erasmus University Rotterdam, Bronder Offset, Rotterdam
21. VRIESENDORP HM, BIJNEN AB, WESTBROEK DL, et al: 1977 Transplant Proc 9 : 293
22. VRIESENDORP HM, GROSSE-WILDE H, DORF ME: 1977, p 129 In Götze, D (ed), Springer Verlag, New York
23. WESTBROEK DL, SILBERBUSCH J, VRIESENDORP HM, et al: 1972 Transplantation 14 : 582
24. WESTBROEK DL, VRIESENDORP HM, VAN DEN TWEEL JG, et al: 1975 Transplant Proc 3 : 427

GENERAL DISCUSSION

7.1. The MHC, its subregions and donor selection

A large number of factors determine whether a kidney transplantation will be a successful operation or not (Kincaid-Smith, 1977). As many kidneys are transplanted yearly, gradually more of these factors are being identified. The quality of the donor kidney for instance appears to be a significant factor, as donor age (Kissmeyer-Nielsen and Kristensen, 1977) and method of kidney preservation (Opelz and Terasaki, 1977[f]) are both reported to influence renal allograft survival rates. The capability of the recipients immune system to amount an immune response against the allograft and the immunization status of the patient are also important parameters (van Hooff et al., 1972; Opelz et al., 1973). Finally of course the degree of histoincompatibility between donor and recipient is important, as it determines the amount of immunosuppressive drugs needed to prevent the rejection reaction (§1.2.3).

Compatibility for the gene products of the MHC is of an enormous importance for renal allograft prognosis, at least when donor and recipient are related (van Rood et al., 1967; Singal et al., 1969; Brynger et al., 1977; Opelz et al., 1977[c]; 1977[e]; Solheim et al., 1977). In sibling donor-recipient combinations compatibility for all gene products of the MHC can be achieved by matching donor and recipient for only some of these gene products, namely by matching for the SD and LD antigens. However, the MHC contains many, maybe hundreds, of loci; only the gene products of a dozen of the loci of the MHC can presently be identified in man (e.g. Bach and van Rood, 1976). Until now mainly the relevance of matching for SD and LD antigens for cadaver kidney transplantation has been studied. Matching for the SD antigens slightly improves the overall outcome of cadaver renal allograft survival (van Hooff et al., 1972; Dausset et al., 1974; Scandiatransplant report, 1975; Festenstein et al., 1976; Opelz et al., 1977[a]; Solheim et al., 1977). This effect is more pronounced in some subsets of patients, for instance recipients with circulating antibodies, males and non-blood group O recipients (van Hooff, 1976; Opelz and Terasaki, 1977[f]). Compatibility in the MLR is also associated with a favourable graft outcome (Cochrum et al., 1973; Festenstein et al., 1976; Opelz and Terasaki, 1977[d]).

A more extensive knowledge exists on the role of the various subregions of the mouse MHC (the H-2 complex) with regard to skin graft survival (Klein, 1975). In these species, many different congenic strains are available which

sometimes differ for only one subregion of the H-2 complex. This makes a rather accurate assessment of the role of the various subregions of the H-2 complex possible. It appears that at least 3 different subregions of the mouse MHC have important histocompatibility effects, namely the K, I and D region. The possibility that other subregions contain weak histocompatibility antigens cannot be excluded.

In larger species a detailed knowledge of the role of the subregions of the MHC is more difficult to obtain. Congenic strains are not available. The information has to be gathered by grafting rarely occurring MHC recombinants and by grafting unrelated individuals with defined MHC differences. However, a strong linkage disequilibrium exists between various defined genes of the MHC in many species. Thus possibly a linkage disequilibrium also exists between defined and undefined genes of the MHC. Consequently, if donor and recipient are matched for defined MHC antigens, they may also be compatible for as yet undefined antigens. Thus, the effect of histocompatibility matching in unrelated donor-recipient combinations has to be interpreted with care, as a prolongation of graft survival obtained by matching for defined MHC antigens may or may not be caused by unintentional matching for undefined MHC antigens. The results of renal allografting in man are difficult to analyze because of the many uncontrollable variables which may play a part, such as different drug protocols, different status of pre-existent immunization, graft loss from technical reasons, differences in crossmatch techniques and inaccurate typing of the donor. Many factors now are known to influence the results (Kissmeyer-Nielsen and Kristensen, 1977). However, many more variables probably are still to be discovered. Therefore studies in a well defined preclinical animal model, performed under standard conditions in one laboratory, may help to solve questions which are difficult to answer in humans.

Previous experiments in dogs (Westbroek et al., 1975) and rhesus monkeys (van Es et al., 1977) have shown the superiority of LD matching over SD matching with regard to renal allograft survival. In this thesis, the importance of compatibility for MLR and for the whole MHC to renal allograft survival has been further explored in dogs. The influence of immunosuppressive therapy has been deliberately excluded by omitting immunosuppressive drugs in any of the studies.

7.2. MLR and donor selection in dogs

7.2.1. MLR and renal allograft survival in dogs

Compatibility for defined as well as for undefined antigens may be important for renal allograft survival. The experiments reported in chapter 6 of this thesis

show that matching for SD antigens is of little influence when donor and recipient are unrelated mongrels and when no immunosuppressive therapy is used. In the same model, compatibility for MLR significantly improved renal allograft survival. Also the studies in recombinant related beagles suggest a predominant role of the LD loci for graft rejection. The possibility that not the LD loci themselves but other loci, closely linked to DLA-D and in linkage disequilibrium with this locus, are relevant for graft survival, cannot be excluded.

The prolongation of renal allograft survival, obtained by matching for MLR, in unrelated mongrels was, though significant, only slight when compared with matching for the whole MHC in littermate beagles (chapter 5). Several reasons may account for the shorter survival times in unrelated MLR negative mongrels compared to sibling beagles. In the first place, kidney allograft survival has not been studied in unimmunosuppressed DLA identical sibling mongrels. It is very well possible, that mongrels are more heterogeneous than beagles and that consequently the cumulative effect of differences for non-MHC histocompatibility systems is stronger in related and unrelated donor-recipient pairs in mongrels than in beagles. This is suggested by the longer kidney allograft survival times in SD and LD identical non-sibling beagles than in mongrels (chapter 5). If this is true, then the kidney allograft survival times in DLA identical sibling mongrels can expected to be shorter than in the comparable beagle group. Thus, the difference in survival times between MLR negative unrelated and sibling mongrel donor-recipient pairs would be less. A second possibility which might explain why the effect of matching for MLR in the unrelated donor-recipient pairs is only slight is that the sensitivity of the used MLR technique was insufficient. It is shown in the next paragraph, that this explanation is improbable. Finally other genetic systems (which may be located within the MHC, outside the MHC on the chromosome which carries the MHC, or on other chromosomes) may exert a considerable influence on renal allograft survival. These possibilities are discussed in § 7.3 and § 7.4.

7.2.2. Applications of the MLR

The MLR can serve different purposes. In the first place, the assay can be used to test the presence of a difference for MLR antigens. If in vitro cellular proliferation and blast formation occurs, the test outcome is called positive. It means that responder and stimulator carry different MLR antigens. If no in vitro proliferation and blast formation occurs, the test outcome is called negative. Then no detectable LD differences are present between responder and stimulator. From the studies described in chapter 3 it appeared, that in our technique, for an unambiguous qualitative discrimination between a negative and a positive test,

harvesting should be performed relatively late in culture, preferably on the sixth or seventh day. When harvesting is performed earlier in culture, false negative reactions may occur.

Secondly, the MLR may be used to measure the strength of the antigenic difference between responder and stimulator. For such a quantitative use of the test harvesting should be preferably performed earlier in culture, namely on the fourth or the fifth day of culture in our technique. With such a relatively short incubation period test reproducibility is better than with a longer incubation period; furthermore the results probably reflect the genetic differences between responder and stimulator more accurately when a shorter culture period is used. In the experiments described in chapter 6, the MLR's were harvested on day 6 or 7. Thus a sufficient discrimination between negative and positive combinations was made. The MLR's in the SD and LD identical donor-recipient combinations were completely negative. Thus, no better correlation between MLR and renal allograft survival would be expected, if a more quantitative MLR test were used.

7.2.3. LD typing methods

The MLR can also be used for the determination of LD phenotypes. LD typing may be helpful for the selection of MLR compatible individuals. For this purpose homozygous typing cells (HTC's) are required. A one way negative reaction of the cells of the tested individual with a reference cell which is homozygous for a given LD determinant indicates that this individual carries that LD determinant too. When population studies with HTC's are performed in humans, a dichotomy of high and low responses against various typing cells is observed (Grosse-Wilde et al., 1975[a]; Keuning et al., 1975[b]). Completely negative reactions are scarcely seen. Therefore in these studies it is assumed that individuals carry a given LD determinant when they show a one way low positive reaction with the typing cell. Such a low one way reaction is called a *typing response*. The results of LD typing with HTC's both in man (van Rood, 1978; Keuning, 1978) and dogs (chapter 4) is disappointing as individuals with an identical LD typing are frequently MLR positive.

Typing for MLR using homozygous reference cells is a time consuming and cumbersome method. Other methods are being developed to replace this test. One method is the *primed lymphocyte test (PLT)* (Sheehy et al., 1975). For this test in vitro sensitized lymphocytes are used as responder cells. Upon restimulation with the same antigen, which was used for primary sensitization, an accelerated response is obtained. This test has three advantages over the conventional MLC typing method. It takes only 24-48 hours to determine the LD phenotype

of an individual. Furthermore, with this method other cells, such as kidney cells or tumour cells which cannot react themselves by a specific proliferation in a mixed culture, can be typed too. Additionally, LD determinants can be recognized with this method, which cannot be recognized with the HTC method (Bradley et al., 1977). Preliminary results with the PLT in dogs indicated that the test may become a useful tool for LD typing in this species (Lansdorp, personal communication). The possible role of the PLT for donor selection still has to be investigated.

Typing for *B cell* antigens or *Ia like* antigens may be another method to select LD identical individuals. Some of these B cell antigens are highly associated with LD antigens or may even be identical to them (van Rood et al., 1978; Terasaki et al., 1977). B cell typing is well developed in mice, man and rhesus monkeys (e.g. Shreffler and David, 1975; van Rood et al., 1977; Balner et al., 1977), but not in dogs. Individuals which are identical for B cell antigens are more frequently MLR negative than individuals which carry different B cell antigens. However an MLR of B cell antigen identical individuals can be strongly positive (van Rood, 1978). B cell typing is done by serological methods. Therefore, B cell typing can be used as a matching procedure for cadaver kidney transplantation.

The need for new methods to select LD identical individuals for transplantation experiments is probably less urgent in dogs than in other species. In the first place, the strong linkage disequilibrium between SD genes and LD genes has the consequence that SD matched unrelated individuals are often automatically LD compatible. Furthermore, the short generation time of the dog allows breeding of homozygous reference dogs of all wanted LD types. However, the development of new techniques for LD typing may still be useful for a further elucidation of the genetics of the MLR and a better understanding of its role in transplantation immunology.

7.2.4. Genetics of the MLR in dogs

The family studies described in chapter 4 and chapter 6 indicate that the canine MHC contains at least two loci which control the outcome of the MLR. In the mouse, at least five loci of the H-2 complex govern the outcome of the MLR (Meo et al., 1973). Thus it can be expected, that in analogy with the mouse model, more MLR loci will be discovered in the dog. Unfortunately, recombinations in the canine MHC are only scarcely found. Therefore, such detailed knowledge on the control of the MLR in dogs is difficult to obtain.

Population studies demonstrated the large polymorphism of LD systems in dogs (van den Tweel et al., 1974; Grosse-Wilde et al., 1975b). Eleven different homozygous typing cells are presently available (chapter 4). Gene frequencies

Table 7.1. Gene frequencies and linkage associations of presently recognized LD types as calculated by Grosse-Wilde et al. (1975[b])

		% occurrence on independent haplotypes		Δ's with SD antigens				
LD type	Source	Mongrel N=64	Beagle N=66	Mongrel		Beagle		frequently associated SD haplotypes
50	Munich	14	15	-	50-4 : .561[c]	50-2 : .542[c]	50-4 : 1.170	2- 4
51	Munich	6	15	-	-	51-2 : 1.148	51-5 : 1.285	2- 5
52	Munich	6	26	-	52-4 : .439	52-9 : 1.561	52-6 : 1.912	9- 6
53	Munich	14	9	53-3 : .812	-	53-3 : .923	-	3- —
54	Rotterdam	12	0	54-10: .625	54-5 : .547[c]	-	-	10- 5
55	Rotterdam	31	3	55-3 :1.894	55-11: .488[c]	-	-	3-11
56	Munich	NT[a]	14[b]			56-1 : .909	56-13: .909	1-13
57	Paris	NT	NT					8-13
58	Rotterdam	NT	NT					9- 4
R1	Rotterdam	NT	NT					7-13
R2	Rotterdam	NT	NT					7- —

[a] NT not tested
[b] 29 haplotypes studied
[c] p-value > 0.05

and delta values for linkage disequilibrium were calculated for only part of them, by Grosse-Wilde (1975[a]). Table 7.1 presents some gene frequencies and linkage associations in beagles and mongrels. No population studies were performed with DLA-57, DLA-58, R1 and R2. These latter typing cells were used to type families for the detection of recombinations which otherwise might not have been demonstrated.

It should be realized that in dogs almost all available typing cells are homozygous by descent. This implies that they are homozygous for both MLR loci. Thus the "LD type" which they represent may be the combination of the products of two loci. In other words, with the use of the presently available typing cells, typing will be often done for the products of two loci of an individual by means of one typing cell. On the other hand, if an individual carries one LD determinant of the typing cell, but not the other, probably no one way negative reaction will be seen.

Consequently, an efficient analysis of the population genetics of the separate LD loci each and their relevance to homografting can only be made if reagents are available which react with the products of only one locus. At present, such reagents are not available for dogs. Theoretically, they can be obtained by three different methods:

a) a search for homozygous reference cells, which are "empty" at one locus,
b) the generation of PLT cells against siblings with a recombination in the MHC and
c) the development of antisera against the gene products of one locus.

When such reagents become available this may have important consequences. For instance, it might appear that the gene products of one locus (e.g. the DLA-D locus) are more important for donor selection than the gene products of other LD loci. If the two locus model would be applicable to man, than it could provide a possible explanation for the fact that some kidneys are not rejected in spite of a strongly positive MLR (e.g. Opelz and Terasaki, 1977[d]). Identity for the gene products of the LD locus which is important for allograft survival could be masked by a concomitant difference for the LD locus which is not relevant for allograft survival.

It is disappointing that in spite of all efforts to develop methods to select MLR compatible unrelated donor-recipient pairs, only a slight effect of matching for MLR on renal allograft survival is seen in our experiments. However, it is possible that matching for MLR becomes more important when the recipients are treated with immunosuppressive drugs. All the same one has still to take into account the possibility that besides the LD systems many other histocompatibility systems exist, which collectively determine whether a kidney homograft will be rejected or not.

7.3. Other genetic systems of the MHC and donor selection in dogs

7.3.1. Serologically defined antigens

The SD antigens are probably the best defined antigens of the MHC in dogs. Typing for these antigens is of the utmost importance for the selection of defined related donor-recipient combinations. Unfortunately identity for only these antigens in the absence of a negative MLR does not prolong allograft survival when the donor is unrelated (chapter 6). Nevertheless the SD antigens are very useful markers for the selection of unrelated donor-recipient pairs because of the very high linkage disequilibrium between the SD and LD antigens in dogs (van den Tweel et al., 1974; Grosse-Wilde et al., 1975[b]; own unpublished observations).

Matching for SD antigens in man significantly improves cadaver donor renal allograft survival (chapter 1, § 1.4.2). This observation does not contradict the observation in dogs that SD matching in LD mismatched individuals does not prolong graft survival (chapter 6). In this group of dogs, the concomitant mismatch for LD antigens considerably reduces the chances of an accidental match for transplantation antigens which can be in linkage disequilibrium with the LD antigens. In the rhesus monkey similar results have been obtained, i.e. SD identity is not associated with a prolonged kidney allograft survival in contrast to LD identity (van Es et al., 1977). The experiments should be repeated with the use of immunosuppressive drugs for a more definitive assessment of the value of identity for SD antigens alone for renal allograft survival.

7.3.2. Other defined markers of the canine MHC

Studies with bone marrow transplantation in dogs suggested the existence of a separate gene which determines allogeneic resistance against bone marrow grafts (Vriesendorp et al., 1975[b]). This gene is in all probability located between the SD and the LD genes of the DLA complex. No in vitro assay for the determination of this genetic trait is yet available.

As in humans, the locus for the polymorphic enzyme marker phosphoglucomutase-3 (PGM_3) in dogs is linked to the MHC (Meera Khan et al., 1978) The exact localization of the PGM_3 locus is unknown but it has to be located outside the DLA complex. No role for donor selection of this marker has yet been demonstrated.

So far, attempts to identify Ia genes in dogs have been unsuccessful (Vriesendorp et al., 1977[b]). Renal allografts performed in rhesus monkeys in Ia identical MLR positive donor-recipient pairs did not reveal an important influence of compatibility for these genes alone on allograft survival (Balner, personal communication). Because of the similarities between the MHC of rhesus monkeys

and the canine MHC no separate role of compatibility for Ia antigens for graft survival would be expected in the comparable dog model.

Many more markers have been identified in the human MHC. Some of them, which are located outside the MHC, may be useful to detect recombinations outside the HLA complex (Bach and van Rood, 1976). It may thus become possible to assess the relevance of parts of the chromosome outside the HLA complex for renal allograft survival. Except for PGM3 no such markers are yet identified in dogs.

7.4. Minor histocompatibility systems in dogs

7.4.1. Relevance for renal allograft survival

The experiments in non-sibling DLA matched beagles (chapter 5) suggest that the cumulative strength of non-MHC incompatibilities can be comparable in strength to MHC incompatibilities. This observation is in agreement with skin grafting experiments in mice (Graff et al., 1966). In that model, the cumulative effect of minor histocompatibility antigens appeared as strong as MHC differences in some MHC identical donor-recipient combinations. Such minor histocompatibility systems, each of which may be weak, become only important in the absence of other, stronger histocompatibility differences. Similar observations were done in a retrospective analysis of human related donor-recipient pairs by Opelz and Terasaki (1977[e]). They calculated that, in related donor-recipient combinations, differences for minor histocompatibility antigens cause a 15% kidney allograft failure rate within one year. Consequently it can be postulated that the same minor histocompatibility differences would cause higher, maybe even 30%, yearly kidney allograft failure rates in unrelated individuals. The resulting 70% one year graft survival in MHC identical unrelated individuals would be equal to the observed one year graft survival in one haplotype different related donor-recipient pairs. This would be in perfect agreement with our experiments in non-sibling beagles where kidney allograft survival was also comparable to kidney allograft survival in one haplotype different related donor-recipient pairs (chapter 5).

The similarity in the results obtained in beagles and humans is conspicuous in view of the differences between the experimental model and the clinical situation. In the first place, no immunosuppression is used in the beagle model, and no proof is available that non-MHC differences and MHC differences are equally well overcome by immunosuppression. In fact Silvers et al. (1967) found that the response to MHC antigens in rats was more difficult to control by immunosuppression than the response to non-MHC antigens. However, the general validity of the hypothesis, "that the weaker the antigens, the more easily immunosup-

pression subdues recipient responsiveness", is questioned by Hildemann (1970), and no evidence is available that differences for weak histocompatibility systems can easily be suppressed if their cumulative effect is strong. Secondly, our recipients did not receive blood transfusions prior to transplantation, in contrast to most human kidney recipients. This is an important point, as blood transfusions administered prior to renal allografting may have an important influence on renal allograft survival in man (Opelz et al., 1974b; Persijn et al., 1977), rhesus monkeys (van Es et al., 1977) and dogs (Obertop et al., 1978). Thirdly, our dog recipients were not uremic at the time of transplantation, but it is unknown to what extent this point might influence the results.

Thus, the similarity in results in beagles and humans can be caused by chance. New experiments in a more clinical set-up should provide more definite information on the relevance of minor histocompatibility systems to renal allograft survival.

7.4.2. Number of minor histocompatibility systems

The number of minor histocompatibility systems and their alleles, which are involved in the genetic control of renal allograft survival in beagles, appears to be relatively small (chapter 5). This is rather unexpected in view of the large number of minor histocompatibility systems that are reported to be involved in skin allograft survival in mice (Graff and Bailey, 1973). Two reasons however can be indicated here which may explain this difference. In the first place, the polymorphism in beagles may be limited as this is a selected breed; thus the number and polymorphism of minor histocompatibility systems may appear to be greater in mongrels for instance. Secondly, it may be that different minor histocompatibility systems are involved in the control of skin and kidney allograft survival. An indication for the existence of organ specific differences in dogs are the differences in survival times of DLA identical skin and kidney allografts (see also chapter 1, § 1.7.).

The effect of minor histocompatibility differences can be overcome by immunosuppressive therapy. In addition, the administration of blood from third party blood donors to allograft recipients prior to transplantation considerably improves renal allograft survival in dogs which are treated with immunosuppressive drugs (Obertop et al., 1978). Therefore it will be important to study how many and which histocompatibility systems are important under those conditions.

7.4.3. Matching for minor histocompatibility systems

Compatibility for antigens of minor histocompatibility systems can theoretical-

ly contribute to improved survival rates of renal homografts. Matching for such antigens can only be performed when techniques and reagents are available for the recognition of the gene products of the alleles of those systems. Several polymorphic genetic systems are being recognized in the dog, such as erythrocyte antigens, canine secretory alloantigens (CSA), polymorphic erythrocyte and leucocyte enzymes, serum proteins and non-DLA serologically defined leucocyte antigens (Joint Report of the Second International Workshop on Canine Immunogenetics, 1976).

The CSA system has many similarities with the blood group ABO system in man (Zweibaum et al., 1974). However, the ABO blood group antigens appeared to be important transplantation antigens (Starzl et al., 1964; van Hooff, 1976; Opelz and Terasaki, 1977[f]). No histocompatibility effect of the CSA system has sofar been demonstrated for graft versus host reactions in bone marrow transplantation (Vriesendorp et al., 1975[b]). A phenotypic difference for the enzyme marker PGM_2 is correlated with a high incidence of graft versus host reactions in bone marrow transplantation in DLA identical dogs (Vriesendorp et al., 1975[b]). Studies of CSA and PGM_2 with regard to renal allografting have not been performed yet. Sex-linked histocompatibility systems appear to be of importance in immunized kidney allograft recipients (Vriesendorp et al., 1978) but not in unimmunized recipients (chapter 5).

The possibility that identity or difference for other markers can influence renal allograft survival, has as yet been insufficiently investigated (Vriesendorp et al., 1977[b]). Furthermore, no systematic attempts have been made to produce reagents which can recognize minor histocompatibility antigens which are relevant for renal allograft survival. One possible approach of this problem may be to select families, in which a short survival of DLA identical kidney allografts is seen. Repeated immunization with cells or tissues from DLA identical donors in such families may hopefully lead to the production of antisera which can help to identify non-MHC differences in those families. Thereafter, population studies with the obtained antisera should be performed to select DLA identical related and unrelated individuals which are matched and mismatched for the studied antigen. Finally renal allografts should be performed in such combinations to test the relevance of matching for the newly defined antigen for renal homografting. Evidently, the difficulty of the logistics of matching unrelated individuals increases with the number and polymorphism of the histocompatibility systems typed for. However, matching for such systems should be possible in beagles after the production of the necessary reagents as the number of important minor histocompatibility systems in that race is probably limited. It is possible, that under adequate immunosuppressive therapy, the number of relevant minor histocompatibility systems is limited in man, as only 10-15% of the HLA identical kidneys from living related donors are rejected. If this were to prove true, match-

ing for minor histocompatibility systems should become feasible, provided that useful reagents can be produced. In the human situation matching for such systems might be especially helpful for recipients with rare HLA phenotypes for whom HLA identical kidneys can not be found.

7.5. Immune responsiveness

7.5.1. "High and low responders"

The one year survival rate of human kidney allografts derived from two HLA haplotypes different sibling donors is about 50% (Opelz and Terasaki, 1977[e]). The overall one year survival rate of cadaver donor kidneys, regardless of HLA matching, is also ± 50% (Human Renal Transplant Registry, 13th report, 1977). Thus many kidneys function during long periods in spite of strong histocompatibility differences. In other words, some individuals *respond* to a renal homograft with a strong rejection reaction, while others do not. Doubtless, all or almost all of these allografts are rapidly rejected when the recipients are not treated with immunosuppressive drugs. Thus, *high responders* may be defined as individuals who do reject the transplanted kidney under immunosuppressive therapy after a short time, while *low responders* do not.

7.5.2. Prospective identification of high and low responders

A prospective identification of high and low responders would be of considerable clinical importance. In the first place, low responders could be given priority to high responders when enough kidneys are not available. Secondly, the donor selection practice would probably be different in both groups. High responders might profit more from HLA matching than low responders (van Hooff et al., 1972; Opelz and Terasaki, 1973). Then high responders should wait for optimally matched kidneys or preferably obtain a kidney from a HLA identical living related donor. In the third place, the needed dosage of immunosuppressive drugs would expected to be higher in high responders than in low responders.

About 50% of the patients do not produce cytotoxic antibodies in spite of 30 blood transfusions (Opelz et al., 1973). Kidney allograft prognosis in patients with antibodies appears to be worse than in patients who do not produce antibodies (van Hooff et al., 1972; Opelz et al., 1973). Thus patients who do produce antibodies after blood transfusions can be probably considered to be high responders. Patients who do reject the first transplanted kidney in a relatively short time, generally reject a second or third kidney after a short time as well. Thus, transplantation of a kidney is an excellent way to determine whether a patient

is a high responder or not (Opelz et al., 1976). Of course, it would be preferable to determine the *responder status* of a patient by in vitro tests beforehand. It seems that low in vitro reactivity of lymphocytes to PHA or allogeneic lymphocytes is correlated with a good renal allograft survival rate (Thomas et al., 1977; Opelz and Terasaki, 1977[d]). However, at present these tests do not yet allow an accurate prospective discrimination between high and low responders.

7.5.3. Immune responsiveness and kidney allograft survival in dogs

Fifteen kidneys transplanted to DLA identical non-sibling beagles were all rejected within fifty days (chapter 5). In another experiment kidneys from DLA identical unrelated donors were transplanted to recipients which had permanently accepted a kidney from a DLA identical sibling donor. It would be expected that a second kidney, derived from a DLA identical donor and transplanted to such a *long survivor,* would be rejected within fifty days too. Contrary to this expectation, two out of five such second transplants were not rejected (own unpublished observations). Furthermore, skin allografts transplanted to these same two recipients and obtained from the second kidney donor, were not rejected either. No immunosuppression was used in these experiments. This is a remarkable observation, as all other skin grafts transplanted in our laboratory, even from DLA identical siblings, were always rejected. The chances that the recipient and donor of the second kidney were identical for all relevant minor histocompatibility systems are small (chapter 5). Thus these animals can be probably considered to be "low responders" even though no immunosuppressive therapy was used. Such a low responder status is not necessarily an inherited quality. It is even possible that an active mechanism is involved, e.g. enhancement (see chapter 1, § 1.2.1.). However, a more aspecific mechanism might be suspected to play a role, as kidneys from different donors were permanently accepted by one recipient. Apparently this situation is comparable to the situation in man, where a good correlation between the survival times of first and subsequent kidney homografts is also reported (Opelz and Terasaki, 1976).

After the administration of blood transfusions some dogs produce detectable cytotoxic antibodies, while others do not. A low antibody response was not clearly correlated with a prolonged renal allograft survival in dogs who did not receive immunosuppression (Bijnen et al., 1975[b]; Bull et al., 1978). However, in recipients with immunosuppression a correlation between antibody response and kidney allograft survival was found (Obertop et al., 1978). Thus a useful discrimination between low and high responders seems only possible when the recipients are treated with immunosuppressive drugs.

Studies in which the in vitro immune responsiveness of lymphocytes to a

variety of antigens or mitogens is correlated with renal allograft survival in immunosuppressed dogs still have to be performed.

7.5.4. Genetics of the immune response in dogs

No systematic studies have been performed on the genetic background of the immune response against allografts in dogs. In fact it is uncertain, to what extent the responder status is an inherited or an acquired quality. Preliminary studies indicate that the immune responses against the copolymers of L-glutamic acid with L-alanine (GA), L-lysine (GL) and L-tyrosine (GT) are under control of genes linked to the DLA complex (Vriesendorp et al., 1977[b]). The role of these genes in transplantation immunity is unknown. A low immune response against GT does not necessarily imply prolonged kidney graft survival. On the contrary, in the experiment described in chapter 6, the GT non-responder rejected the kidney much faster than the GT responder.

The immune responsiveness to blood transfusions could be genetically determined (Bull et al., 1978). However, if this phenomenon is under genetic control, it is not governed by a simple Mendelian system linked to, or within, the DLA complex (Bijnen et al., 1975[b]; Bull et al., 1978). Thus it is likely that the response to a whole organ graft is at least as intricate.

7.6. Non genetic influences on renal allograft survival

Biologic variation plays an important role in kidney allograft survival in inbred rat strains (Tinbergen, 1971). Likewise, the biologic variation may be considerable in the dog model too. The preoperative administration of blood transfusions significantly prolongs renal allograft survival in immunosuppressed recipients (Obertop et al., 1978). This may be due to an aspecific effect of blood transfusions; genetic factors are not known to play a part. The impact of immunosuppression on the different other factors influencing graft survival is incompletely known and may be complicated. Conclusions drawn from models where no immunosuppression is used are not necessarily valid for a similar model with immunosuppression. The addition of immunosuppressive drugs to experimental protocols however may induce other variables, as some dogs for example may be more sensitive for immunosuppressive drugs than others. These and other variables have to be taken into account when analyzing the results of allografting experiments. Variability however, can be minimized by conducting experiments in one laboratory under standard conditions in a standardized model.

7.7. Extrapolation to human kidney transplantation

Extrapolation of observations in experimental animals to the human situation is hazardous. An exact imitation of the clinical situation in an animal model is impossible. Species specific differences may be the main reason that principles established in an experimental animal are not applicable to man. However, if an experimental model has many similarities with the clinical situation, it becomes more likely, that additional observations in such a model have their analogues in man too. Especially when similar principles are uncovered in different preclinical experimental animal models in quite distant species, for instance dogs and rhesus monkeys, it becomes likely that they are valid in man too. For instance random blood transfusions, administered to recipients prior to transplantation, considerably prolongs renal allograft survival in immunosuppressed rhesus monkeys and dogs (van Es et al., 1977; Obertop et al., 1978). These findings in both models probably justify the administration of blood to patients waiting for a renal allograft on dialysis. The dog model is immunogenetically in many respects similar to man (e.g. Vriesendorp et al., 1977[b]). Consequently observations in this model may very well be valid for man too.

Extrapolation of the observations in this thesis implies that when a choice can be made from different living related donors, an MHC identical donor should be selected. If the recipient has a recombination within the MHC, an MLR identical donor should be chosen. For cadaver kidney transplantation HLA-D identical donor-recipient pairs are to be preferred. The selection of completely MLR identical unrelated individuals will be difficult because of the complex genetic control of the MLR. For a further improvement of kidney survival rates attempts should be undertaken to match for non-HLA histocompatibility systems, which may appear very important in unrelated donor-recipient combinations.

The overall result of histocompatibility matching is not only determined by the improvement in kidney survival rates procured by a better match grade, but is also dependent on the fraction of the recipient pool which can profit of such a better match grade. Well matched recipients for each donor are more likely to be found in larger recipient pools. Thus the effectiveness of histocompatibility matching programms can be improved by extending these recipient pools. In larger geographical areas however a greater heterogeneity of genetic systems may be expected. Thus when kidneys are distributed over larger geographical areas, identification of still undefined histocompatibility systems becomes even more important. Methods for the identification of such systems may be developed in an experimental animal as the dog.

7.8. Future histocompatibility research in the dog

To obtain a more complete insight into the genetic factors which are relevant for renal transplantation the following subjects should be taken under study:

a) development of B cell typing and PLT in dogs
b) development of methods for the identification of minor histocompatibility structures
c) development of methods for the prospective identification of high and low responders
d) testing the relevance of matching for the different histocompatibility systems for renal allograft survival in recipients under immunosuppressive therapy
e) analysis of the factors which cause a prolonged kidney allograft survival in recipients which received blood transfusions prior to transplantation, and
f) testing the relevance of matching for the different histocompatibility systems for renal allograft survival in immunosuppressed recipients which received blood transfusions prior to transplantation.

It has become obvious that the most rapid way to define optimal conditions for organ allografting is to intensify transplantation research in immunologically well defined experimental animals. Some of these studies are in progress in our laboratory. However, it is clear, that the formidable task outlined above, is too large for one laboratory. Joint efforts of several laboratories with a co-ordinated programm are required to complete this duty.

SUMMARY

The fate of a transplanted organ is dependent, among other factors, on the antigenic differences between organ donor and recipient. Selection of compatible donor-recipient combinations is done by means of tissue-typing.

In chapter 1 the basic aspects of the technique and the genetics of two different tissue-typing methods, namely a serologic method, the lymphocytotoxicity test and a cell culture method, the mixed lymphocyte reaction (MLR), are reviewed. It appears from the literature on human kidney transplantation that a good correlation between renal allograft survival rates and serological compatibility is found when donor and recipient are related. The correlation between kidney homograft survival rates and serological match grade is less good when donor and recipient are not related. Most authors report that kidneys from MLR compatible donors do significantly better than kidneys from MLR incompatible donors. However a precise evaluation of the value of matching for MLR antigens is hampered by the retrospective character of most studies and the fact that different MLR techniques and criteria have been used. Furthermore, the correlation between kidney allograft survival and compatibility for MLR is not complete, as some kidneys do well in spite of a strongly positive MLR, while some other kidneys, derived from MLR compatible donors, are rejected in a short time. In most experimental studies in animals and man a positive correlation between MLR compatibility and allograft survival is found.

The purpose of the experiments described in this thesis was to obtain a more accurate insight into the possible role of matching for MLR antigens and other antigens for donor selection for renal transplantation. The dog was chosen as an experimental animal because of its immunogenetical similarities to man and the extensive knowledge, which is available on its major histocompatibility complex (MHC).

A more extensive description of the used techniques is presented in chapter 2. The technique which was used for the MLR is described extensively, as the MLR has a central role in the studies reported in this thesis.

For an optimal evaluation of the correlation between MLR compatibility and organ allograft prognosis, knowledge of the kinetics of the MLR is required. The outcome of the MLR is dependent on the genetic disparity between the two tested individuals, but more variables determine the test result. In chapter 3 the influence of variation of the used numbers of responder cells, stimulator cells, and of the responder/stimulator cell ratio is studied in genetically different cell

combinations. The number of responder and stimulator cells and their ratio which produces an optimal stimulation in MLR appear to be independent of the genetic disparity between the stimulator and responder cells. The optimal culture length is variable too. It appears to be shorter in unrelated cell combinations. The observed variability hampers a quantitative interpretation of the test; for a quantitative interpretation a short culture length should be chosen (4 or 5 days); however when such a short culture length was used, false negative results were sometimes obtained.

The genes which determine the outcome of the MLR are located within the MHC. The recombination frequency between the MLR genes and the serologically defined genes of the canine MHC is estimated to be 0.007 (chapter 4). The calculation of this estimate is based on the assumption that the canine MHC contains only one MLR locus. It is argued, however, that the canine MHC probably contains at least two MLR loci. Presently eleven different homozygous reference cells are available to type for the products of the MLR genes. Individuals which are identical for the MLR antigens according to MLR typing frequently stimulate each other in MLR, although less frequent than individuals with a different MLR typing. Consequently this typing method with homozygous reference cells appears not very useful for donor selection.

In chapters 5 and 6 the results are presented of renal allografting experiments in dogs in various genetically different donor-recipient combinations. Immunosuppressive drugs were not used. It appears that not only MHC systems determine the outcome of renal allograft survival. Important non-MHC systems must be assumed which also influence graft survival. In the beagle, the number of non-MHC systems and their polymorphism seems to be small. Within the MHC, the subregions which contain the MLR genes play a predominant role in the genetic control of kidney allograft survival. This is apparent from the renal allografting experiments with related beagles in which the donor or recipient has a recombination in the MHC. Furthermore, in mongrels a prolonged graft survival is only observed in MLR compatible donor-recipient combinations; compatibility for serologically defined antigens alone has no favourable influence on graft survival.

In chapter 7 (general discussion), the different factors which together determine the ultimate fate of a transplanted kidney are reviewed. Histocompatibility differences are very important. The impact of a number of different methods for the determination of histocompatibility differences is discussed. Many antigens are important for graft survival, but only some of them are presently defined. In the dog only the importance of MLR antigens for allograft survival has been demonstrated. A direct MLR between donor and recipient is presently the only reliable method for the selection of MLR compatible dogs. The number and polymorphism of the undefined histocompatibility antigens in beagles may be

small. Therefore it is attractive to develop methods to type for these antigens. As the dog is immunogenetically very similar to man, it is probable that matching for these non-MHC systems can improve transplant survival rates in man too. Some individuals react more strongly to histocompatibility differences than others. Prospective identification of "responders" would be an additional approach to improve the results of kidney transplantation programms. In vitro methods for such a prospective identification of responders are not yet available in dogs.

The results described in this thesis demonstrate that in unrelated unimmunosuppressed dogs kidney allograft survival times can be improved by the selection of an MLR compatible donor. A detailed knowledge of the kinetics and the genetics of the MLR in dogs as described in this thesis may lead to a more optimal selection procedure. For a further improvement of kidney allograft survival times attempts should be undertaken to match for other, presently undefined, histocompatibility systems, which in part do not belong to the MHC.

Additional studies have to be performed to determine whether the observations obtained in an experimental model without immunosuppression can be extrapolated to models with immunosuppression and the clinical situation.

SAMENVATTING

De prognose van een getransplanteerd orgaan is beter naarmate de antigene structuur van het transplantaat beter overeen komt met dat van de ontvanger. Selectie van passende donor-ontvanger combinaties geschiedt door middel van weefseltypering.

In hoofdstuk 1 worden de basale technische en genetische aspecten van twee vormen van weefseltypering, namelijk door middel van serologisch onderzoek en door middel van gemengde lymphocyten kweken (mixed lymphocyte reactions, MLR's) besproken. Uit de literatuur blijkt dat er bij de mens een goede correlatie bestaat tussen niertransplantaat overleving en serologische compatibiliteit, wanneer donor en ontvanger verwant zijn. Wanneer donor en ontvanger niet verwant zijn is dit in veel mindere mate het geval. De meeste auteurs vinden dat compatibiliteit voor MLR leidt tot een significant betere transplantaatoverleving. Een nauwkeurige evaluatie van het belang van compatibiliteit voor MLR antigenen wordt bemoeilijkt door het retrospectieve karakter van de meeste studies en het gebruik van verschillende technieken en criteria. De correlatie is voorts niet absoluut, aangezien lange transplantaatoverlevingen worden gevonden ondanks een duidelijke incompatibiliteit voor MLR en daarnaast MLR compatibele nieren soms snel worden afgestoten. In de meeste experimentele onderzoeken bij dieren en mensen wordt echter wel een positieve correlatie gevonden tussen MLR compatibiliteit en transplantaatoverleving.

Het onderzoek dat in dit proefschrift wordt beschreven, heeft tot doel inzicht te verkrijgen in de mogelijke rol, welke "matching" voor MLR antigenen en andere antigenen kan spelen bij donorselectie voor niertransplantatie. Het proefdier dat gebruikt werd, is de hond, omdat er een uitgebreide kennis bestaat van het "Hoofd Histocompatibiliteits Complex" (Major Histocompatibility Complex, MHC) van de hond en dit proefdier in immunogenetisch opzicht veel overeenkomsten vertoont met de mens. De gebruikte technieken worden meer in detail beschreven in hoofdstuk 2, met name de technische uitvoering van de MLR, omdat deze in dit proefschrift een centrale rol speelt.

Voor een optimale evaluatie van de relatie tussen MLR compatibiliteit en transplantaatprognose is kennis van de kinetica van die test gewenst. De uitkomst van de MLR is, behalve van het genetisch verschil tussen twee individuen, afhankelijk van een aantal variabelen. In hoofdstuk 3 wordt de studie beschreven, waarbij de invloed van variatie van het gebruikte aantal respondercellen, het gebruikte aantal stimulatorcellen en de verhouding tussen deze twee celpo-

pulaties wordt onderzocht in genetisch verschillende combinaties. Het aantal responder- en stimulatorcellen en hun ratio, waarbij een optimaal resultaat wordt bereikt, blijkt variabel en niet duidelijk afhankelijk van de genetische verschillen tussen stimulator- en respondercellen. De optimale kweekduur is ook variabel en lijkt korter in onverwante combinaties. De gevonden variabiliteit bemoeilijkt een kwantitatieve interpretatie van de test; deze is waarschijnlijk het meest betrouwbaar bij een korte kweekduur, bijvoorbeeld 4 of 5 dagen; soms wordt bij een zo korte kweekduur echter een vals negatieve uitslag verkregen.

De MLR genen maken deel uit van het MHC. De recombinatiefrequentie tussen de MLR genen en de serologisch gedefinieerde genen van het MHC van de hond wordt geschat op 0.007 (hoofdstuk 4). Bij de berekening van deze schatting werd ervan uitgegaan dat zich in het MHC van de hond slechts één belangrijk MLR locus bevindt. Het MHC telt echter waarschijnlijk tenminste twee MLR loci. Momenteel zijn er elf verschillende homozygote referentiecellen beschikbaar om te typeren voor de producten van de MLR genen. Individuen met een identieke "MLR typering" stimuleren elkaar minder vaak in MLR dan individuen met een verschillende "MLR typering", maar de MLR is toch vaak positief. Het nut van de genoemde typeringsmethode met homozygote typeercellen voor donorselectie lijkt dus betrekkelijk.

In de hoofdstukken 5 en 6 worden de resultaten beschreven van enkele niertransplantaat-experimenten in een aantal genetisch verschillende donor-ontvanger combinaties bij honden. Geen immunosuppressieve middelen werden toegediend. Het blijkt dat men moet veronderstellen dat er buiten het MHC ook andere genetische systemen aanwezig moeten zijn, die de transplantaatoverleving in belangrijke mate beïnvloeden. In de beagle lijkt het aantal van deze non-MHC systemen en hun polymorfisme beperkt. Binnen het MHC zijn de subregionen, die de MLR genen bevatten, van predominant belang voor de transplantaatprognose. Dit blijkt uit de transplantaatoverlevingen van verwante donor-ontvanger combinaties, waarvan donor of ontvanger een recombinatie in de MHC heeft. Bovendien wordt bij onverwante bastaarden alleen een verlenging van de transplantaatoverleving gezien indien donor en ontvanger MLR compatibel zijn, en niet wanneer zij alleen serologisch compatibel zijn.

In hoofdstuk 7, de algemene discussie, worden de verschillende factoren, welke het uiteindelijk al of niet slagen van een transplantatie bepalen, besproken. Histocompatibiliteitsverschillen zijn zeer belangrijk. Het belang van verschillende in vitro methodes om histocompatibiliteitsverschillen aan te tonen wordt bediscussieerd. Van de vele voor transplantatie belangrijke antigenen kunnen er slechts enkele worden herkend. Bij de hond is alleen het belang van compatibiliteit voor MLR antigenen voor transplantatie aangetoond. Voorlopig is de enige betrouwbare methode om MLR identieke individuen te selecteren de directe MLR tussen donor en ontvanger zelf. Omdat in beagles het aantal voor transplantatie belangrij-

ke niet gedefinieerde antigenen mogelijk klein is, is het aantrekkelijk methoden te ontwikkelen om deze te bepalen. Aangezien de hond in immunogenetisch opzicht veel overeenkomsten vertoont met de mens lijkt het waarschijnlijk dat ook bij de mens matching voor deze non-MHC systemen de transplantaatprognose zal kunnen verbeteren. Sommige individuen reageren sterker op histocompatibiliteitsverschillen dan anderen. Prospectieve identificatie van "responders" zou een andere manier zijn om de resultaten van niertransplantatie te verbeteren. In vitro methodes voor zo'n prospectieve identificatie van responders zijn echter nog niet beschikbaar in de hond.

De resultaten beschreven in dit proefschrift, tonen dat, althans in het hondenmodel zonder immunosuppressie, de transplantaatprognose in onverwante donor-ontvanger combinaties verbeterd kan worden door een MLR passende donor te zoeken. Een gedetailleerde kennis van de kinetica en de genetica van de MLR zoals deze in dit proefschrift wordt beschreven kan bijdragen tot het ontwikkelen van efficiëntere selectieprocedures. Om de transplantaatprognose verder te verbeteren moet geprobeerd worden om te "matchen" voor andere nog ongedefinieerde histocompatibiliteitssystemen. Dit zijn niet alleen nog ongedefinieerde systemen welke behoren tot het MHC, maar ook andere systemen, welke niet behoren tot het MHC.

Verder onderzoek is noodzakelijk om na te gaan in hoeverre de bevindingen in een model zonder immunosuppressie geëxtrapoleerd kunnen worden naar modellen waarin wel immunosuppressiva gebruikt worden en naar de klinische situatie.

REFERENCES

BACH, F.H. & HIRSCHHORN, V. (1964) Lymphocyte interaction: a potential histocompatibility test in vitro. Science 142, 813-814

BACH, F.H. & VOYNOW, N.K. (1966) One-way stimulation in mixed leukocyte cultures. Science 153, 545-547

BACH, F.H. & ROOD, J.J. VAN (1976) The major histocompatibility complex. Genetics and biology (third of three parts). New Eng. J. Med. of Medicine 295, 927-936

BACH, M.L., BACH, F.H., RASMUS, W., BURKHOLDER, P.M., DUFEK, J., & KAHN, D. (1975) DL-A LD matching: Its prognostic value for kidney allograft survival. Transpl. Proc. 7: 423-425

BAIN, B., VAS, M.R. & LOWENSTEIN, L. (1964) The development of large immature mononuclear cells in mixed leukocyte cultures. Blood 23, 108-116

BALNER, H., D'AMARO, J., TOTH, E.K., DERSJANT, H., & VREESWIJK, W. VAN (1973) The histocompatibility complex of rhesus monkeys: Relation between RhL and the main locus controlling reactivity in mixed lymphocyte cultures. Transpl. Proc. 5, 323-329

BALNER, H. & VAN VREESWIJK, W. (1975) The Major Histocompatibility Complex of Rhesus monkeys (RhL-A): V. Attempts at serological identification of MLR determinants and postulation of an I region in the RhL-A complex. Transpl. Proc. 7 (suppl. 1), 13-20

BALNER, H. (1977) Genetic regions controlling immune responsiveness in primates. Transplant. Proc. 9, 837-845

BELZER, F.O., PERKINS, H.A., FORTMANN, J.L., KOUNTZ, S.L., SALVATIERRA, O., COCHRUM, K.C. & PAYNE, R. (1974) Is HL-A typing of clinical significance in cadaver renal transplantation? Lancet i, 774-777

BOS, E., MEETER, K., STIBBE, J., VRIESENDORP, H.M., WESTBROEK, D.L., VRIES, M.J. DE, NAUTA, J. & ROOD, J.J. VAN (1971) Histocompatibility in orthotopic heart transplantation in dogs. Transpl. Proc. 3, 155-156

BRADLEY, B.A., TERMIJTELEN, A., FRANKS, D. & ROOD, J.J. VAN (1977) Interpretation of data obtained from primed lymphocyte tests (PLT's), Transpl. Proc. 9, 421-424

BRYNGER, H., SANDBERG, L., AHLMEN, J., BITTER-SUERMANN, H., BLOHME, I., LINDHOLM, A. & GELIN, L.E. (1977) Effect of HLA compatibility on primary kidney grafts from living related and cadaveric donors. Transpl. Proc. 9, 479-482

BULL, R.W., VRIESENDORP, H.M., OBERTOP, H., BIJNEN, A.B., JEEKEL, J., GRUYL, J. DE & WESTBROEK, D.L. (1978) The effect of prior third party blood transfusions on canine renal allograft survival. Submitted for publication

BUSCH, G.J., SCHAMBERG, J.F., MORETZ, R.C., STROM, T.B., TILNEY, M.L. & CARPENTER, C.B. (1977) Four patterns of human renal allograft rejection: a cytologic and in vitro analysis of the infiltrate in 24 irreversibly rejected kidneys. Transpl. Proc. 9, 37-42

BIJNEN, A.B., SCHREUDER, I., GILES, C.M., LOS, W.R.T., MEERA KHAN, P., VOLKERS, W.S. & VAN ROOD, J.J. (1975[a]) A study on genetic markers in families with a recombination in the HL-A region. In: Rotterdam Conference (1974) Second International Conference on Human Gene Mapping. Birth Defects: Original Article Series 9, 77-81. The National Foundation, New York

BIJNEN, A.B., VRIESENDORP, H.M., GRUYL, J. DE, URK, H. VAN, MACDICKEN, F. & WESTBROEK, D.L. (1975[b]) Host responses to blood transfusions after transplantation of a DL-A identical kidney. Transpl. Proc. 7, 431-433

BIJNEN, A.B., SCHREUDER, I., MEERA KHAN, P., ALLEN, F.H., GILES, C.M., LOS, W.R.T., VOLKERS, W.S. & ROOD, J.J. VAN (1976) Linkage relationships of the loci of the major histocompatibility complex in families with a recombination in the HLA region. J. of Immunogenetics 3, 171-183

BIJNEN, A.B., SCHREUDER, I., VOLKERS, W.S., PARLEVLIET, J. & ROOD, J.J. VAN (1977) The lymphocyte activating influence of the HLA-A region. J. of Immunogenetics, 4, 1-5

COCHRUM, K.C., PERKINS, H.A., PAYNE, R.O., KOUNTZ, S.L. & BELZER, F.O. (1973) The correlation of MLC with graft survival. Transpl. Proc. 5, 391-396

DAUSSET, J., HORS, J., BUSSON, M., FESTENSTEIN, H., OLIVER, R.T.D., PARIS, A.M.I. & SACHS, J.A. (1974) Serologically defined HL-A antigens and long-term survival of cadaver kidney transplants. A joint analysis performed by France-Transplant and the London Transplant group. New Engl. J. Med. 290, 979-984

DAVID, C.S. (1977) The major histocompatibility system of the mouse. In Götze, D. (ed.) The major histocompatibility system in man and animals. Springer-Verlag, New York, p. 255-312

ES, A.A. VAN, MARQUET, R.L., VREESWIJK, W. VAN, TANK, B. & BALNER, H. (1977) Influence of matching for RhLA (SD) antigens and of mixed lymphocyte reactivity on allograft survival in unrelated rhesus monkeys. Transpl. Proc. 9, 257-260

EIJSVOOGEL, V.P. ROOD, J.J. VAN, DU TOIT, E.D. & SCHELLEKENS, P.Th.A. (1972) Position of a locus determining mixed lymphocyte reactions distinct from the known HL-A loci. Eur. J. of Immun. 2, 413-418

FABRE, J.W. & MORRIS, P.J. (1974) Passive enhancement of homozygous renal allografts in the rat. Transplantation 18, 429-435

FERGUSON, R.M., NOREEN, H., YUNIS, E.J., SIMMONS, R.L. & NAJARIAN, J.S. (1977) Does "Responder" / ..non-responder" status influence renal allograft success? Transpl. Proc. 9, 69-72

FESTENSTEIN, H., ABBASI, K., SACHS, J.A & OLIVER, R.T.D. (1972) Serologically undetectable immune responses in transplantation. Transpl. Proc. 4, 219-222

FESTENSTEIN, H. (1973) Immunogenetic and biological aspects of in vitro lymphocyte allotransformation in the mouse. Transpl. Rev. 15, 62-88

FESTENSTEIN, H., SACHS, J.A., PARIS, A.M.I., PEGRUM, G.D. & MOORHEAD, J.F. (1976) Influence of HLA matching and blood transfusion on outcome of 502 London transplant group renal graft recipients. Lancet i, 157-161

GLUCKMANN, E. (1973) Mixed leukocyte cultures in dogs. A microtechnique using gradient-purified cells. Transplantation 15, 642-645

GOLDMANN, S.F. & FLAD, H.D. (1975) Histocompatibility testing in dogs. I. A semi-micro mixed lymphocyte culture (MLC) technique for histocompatibility matching in dogs. Tissue antigens 5, 145-154

GOTZE, D. (1977) The major histocompatibility system in man and animals. Springer-Verlag, New York

GRAFF, R.J. & BAILEY, D.W. (1973) The non-H2 histocompatibility loci and their antigens. Transpl. Rev. 15, 26-49

GRAFF, R.J., SILVERS, W.K., BILLINGHAM, R.E., HILDEMANN, W.H. & SNELL, G.D. (1966) The cumulative effect of histocompatibility antigens. Transplantation 4, 605-617

GROSSE-WILDE, H., BAUMANN, P., NETZEL, B., KOLB, H.J., WANK, R., MEMPEL, W. & ALBERT, E.D. (1973) One way non-stimulation in MLC related to DL-A homozygosity. Transpl. Proc. 5, 1567-1571

GROSSE-WILDE, H., NETZEL, B., MEMPEL, W., RUPPET, W., BREHM, G., BERTRAMS, J., EWALD, R., LENHARD, V., RITTNER, CH., SCHOLZ, S. & ALBERT, E.D. (1975[a]) Immunogenetics of LD determinants in man. Histocompatibility testing 1975, 526-532

GROSSE-WILDE, H., VRIESENDORP, H.M., NETZEL, B., MEMPEL, W., KOLB, H.J., WANK, R., THIERFELDER, S. & ALBERT, E.D. (1975[b]) Immunogenetics of seven LD alleles of the DL-A complex in mongrels, beagles and labradors. Transpl. Proc. 7, (suppl. 1) 159-164

GRUYL, J. DE, WESTBROEK, D.L., DIJKHUIS, C.M., VRIESENDORP, H.M., MACDICKEN, I., ELION-GERRITSEN, W., VERSCHOOR, L., HULSMANS, H.A.M. & HORCHNER, P. (1973) Influence of DL-A matching, ALS and 24 hour preservation on isolated pancreas allograft survival. Transpl. Proc. 5, 755-759

HAMBURGER, J., CROSNIER, J., DESCAMPS, B. & ROWINSKA, D. (1971) The value of present methods used for the selection of organ donors. Transpl. Proc. 3, 260-267

HILDEMANN, W.H. (1970) Components and concepts of antigenic strength. Transpl. Rev. 3, 5-21

HOOFF, J.P. VAN, STEEN, G.J., VAN DER, SCHIPPERS, H.M.A. & ROOD, J.J. VAN (1972) Efficacy of HL-A matching in Eurotransplant. Lancet ii, 1385-1388

HOOFF, J.P. VAN, HENDRIKS, G.F.J., SCHIPPERS, H.M.A. & ROOD, J.J. VAN, (1974) Influence of possible HL-A haploidentity on renal graft survival in Eurotransplant. Lancet i, 1130-1132

HOOFF, J.P. VAN (1976) The relative importance of HLA matching in kidney transplantation. Thesis Leyden University, Pasmans, Den Haag, the Netherlands

HUMAN RENAL TRANSPLANT REGISTRY (1977) 13th Report. Transpl. Proc. 9, 9-30

JEANNET, M. (1969/1970) Histocompatibility testing using leukocyte typing and mixed lymphocyte culture in kidney transplants. Helvetica Medica Acta 35, 168-179

JEEKEL, J. (1971) Immunological enhancement of skin allo- and heterografts. Thesis Erasmus University, Rotterdam, the Netherlands; Meindema N.V., Delft, the Netherlands

JOINT REPORT OF FIRST INTERNATIONAL WORKSHOP ON CANINE IMMUNOGENETICS (1973) Tissue antigens 3, 145-163

JOINT REPORT OF SECOND INTERNATIONAL WORKSHOP ON CANINE IMMUNOGENETICS (1976) Transpl. Proc. 8, 289-311

JØRGENSEN, F., LAMM, L.U. & KISSMEYER-NIELSEN, F. (1973) Mixed lymphocyte cultures with inbred individuals: An approach to MLC typing. Tissue antigens 3, 323-339

KEUNING, J.J., TERMIJTELEN, A., BLUSSE VAN OUD ALBLAS, A., TWEEL, J.G. VAN DEN, SCHREUDER, I. & ROOD, J.J. VAN (1975a) Typing for MLC (LD). Transpl. Proc. 7, suppl. 1, 35-39

KEUNING, J.J., TERMIJTELEN, A., BLUSSE VAN OUD ALBLAS, A., GABB, B.W., D'AMARO, J. & ROOD, J.J. VAN (1975b) LD (MLC) population and family studies in a Dutch population. Histocompatibility Testing 1975, 533-543

KEUNING, J.J. (1978) Typing for HLA-D. Thesis Leiden University, Pasmans, Den Haag, the Netherlands

KINCAID-SMITH, P. (1977) Variables conditioning the results of cadaveric kidney transplantation. Transpl. Proc. 9, 31-36

KISSMEYER-NIELSEN, F. & KJERBYE, F.E. (1967) Lymphocytotoxic technique. Purification of lymphocytes by flotation. In Curtoni, E.S., Mattiuz, P.L. and Tossi, R.M. (eds): Histocompatibility testing, p 381-383 Munksgaard, Copenhagen

KISSMEYER-NIELSEN, F. & KRISTENSEN, T. (1977) An overview of the HLA system: clinical aspects Transpl. Proc. 9, 1789-1794

KLEIN, J. (1975) Biology of the Mouse Histocompatibility-2 Complex. Springer-Verlag, New York

KOCH, C.T., HOOFF, J.P. VAN, LEEUWEN, A. VAN, TWEEL, J.G. VAN DEN, FREDERIKS, E., STEEN, G. VAN DER, SCHIPPERS, H.M.A. & ROOD, J.J. VAN (1973) The relative importance of matching for the MLC versus the HL-A loci in organ transplants. Histocompatibility testing 1972, 521-524. Munksgaard, Copenhagen

LANGEN, Z.J. DE, JONG, B. DE, EYSINK SMEETS, M., VRIESENDORP, H.M., JERUZALEM, CH. R. & WILDEVUUR, CH.R.H. (1975) Unmodified lung allograft survival related to the major histocompatibility complex in the dog. In: Cachera. J.P. (ed.) Abstracts of 10th Congress European Soc. for Experimental Surgery, p. 166

LENGEROVA, A. (1969) Immunogenetics of tissue transplantation. North-Holland Research Monographs. Frontiers of biology, 16. North-Holland Publishing Company, Amsterdam, the Netherlands

MARQUET, R.L., HEYSTEK, G.A. & TINBERGEN, W.J. (1971) Specific inhibition of organ allograft rejection by donor blood. Transpl. Proc. 3, 708-710

MEERA KHAN, P., LOS, W.R.T., DOES, J.A. VAN DER & EPSTEIN, R.B. (1973) Isoenzyme markers in dog blood cells. Transplantation 15, 624-628

MEERA KHAN, P., VRIESENDORP, H.M., SAISON, R., VOLKERS, W.S., LOS, W.R.T., & DOPPERT, B. (1978) Linkage between PGM_3 and the genes determining the major histocompatibility complex in canis familiaris (the domestic dog). 4th international Workshop on Human Gene Mapping, Birth Defects, Original Article Series, the National Foundation, New York, 1978 In press.

MEMPEL, W., GROSS-WILDE, H., ALBERT, E. & THIERFELDER, S. (1973) Atypical MLC reactions in HL-A typed related and unrelated pairs. Transpl. Proc. 9, 401-407

MEO, T., VIVES, J., MIGGIANO, V. & SHREFFLER, D. (1973) A major role for the Ir-1 region of the mouse H-2 complex in the mixed leucocyte reaction. Transpl. Proc. 5, 377-381

MONACO, A.P., CAMPION, J.P. & KAPNICK, S.J. (1977) Clinical use of antilymphocyte globulin. Transpl. Proc. 9, 1007-1018

NETZEL, B., GROSSE-WILDE, H., RITTNER, CH., PRETORIUS, A.M.G., SCHOLZ, S. & ALBERT, E.D. (1975) HLA/MLC recombination frequency and LD typing in HL-A/MLC/Bf/PGM_3 recombinant families. Histocompatibility testing 1975, 955-959, Munksgaard, Copenhagen

OBERTOP, H., BIJNEN, A.B., VRIESENDORP, H.M. & WESTBROEK, D.L. (1978) Prolongation of renal allograft survival in DLA tissue typed beagles after third party bloodtransfusions and suboptimal immunosuppressive treatment. Submitted for publication

OLIVER, R.T.D., SACHS, J.A., FESTENSTEIN, H., PEGRUM, G.D. & MOORHEAD, J.F. (1972) Influence of HL-A matching, antigenic strength and immune responsiveness on outcome of 349 cadaver renal allografts. 3 Years experience of the London transplant group. Lancet ii, 1381-1384

OPELZ, G., MICKEY, M.R. & TERASAKI, P.I. (1972) Identification of unresponsive kidney transplant recipients. Lancet i, 868-871

OPELZ, G., MICKEY, M.R. & TERASAKI, P.I. (1973) Blood transfusions and unresponsiveness to HL-A Transplantation 16, 649-654

OPELZ, G., MICKEY, M.R. & TERASAKI, P.I. (1974[a]) HL-A and kidney transplants: reexamination. Transplantation 17, 371-382

OPELZ, G. & TERASAKI, P.I. (1974[b]) Poor kidney transplant survival in recipients with frozen blood transfusions or no transfusions. Lancet ii, 696-698

OPELZ, G. & TERASAKI, P.I. (1976) Recipient selection for renal retransplantation. Transplantation, 21, 483-488

OPELZ, G., MICKEY, M.R. & TERASAKI, P.I. (1977[a]) HLA matching and cadaver kidney transplant survival in North America. Influence of center variation and presensitization. Transplantation 23, 490-497

OPELZ, G., MICKEY, M.R. & TERASAKI, P.I. (1977[b]) Calculations on long term graft and patient survival in human kidney transplantation. Transpl. Proc. 9, 27-30

OPELZ, G., MICKEY, M.R. & TERASAKI, P.I. (1977[c]) Influence of race on kidney transplant survival. Transpl. Proc. 9, 137-142

OPELZ, G. & TERASAKI, P.I (1977[d]) Significance of mixed leukocyte culture testing in cadaver kidney transplantation. Transplantation 23, 375-380

OPELZ, G. & TERASAKI, P.I. (1977[e]) Studies on the strength of HLA antigens in related donor kidney transplants. Transplantation 24, 105-111

OPELZ, G. & TERASAKI, P.I. (1977[f]) Factors influencing effect of HLA matching on cadaver kidney transplants. Transpl. Proc. 9, 1795-1797

OTTE, J.B., LAMBOTTE, L., WESTBROEK, D.L., VRIESENDORP, H.M. & HAALEBOS, M.P. (1976) Long term survival after orthotopic liver transplantation in DL-A identical beagles. Eur. Surg. Res. 8, (suppl. 1) 63

PAYNE, R. & ROLFS, M.R. (1958) Fetomaternal leukocyte incompatibility J. Clin. Invest. 37, 1756-1763

PENN, I. (1977) Development of cancer as a complication of clinical transplantation. Transpl. Proc. 9, 1121-1127

PERSYN, G.G., HOOFF, J.P. VAN, KALFF, M.W., LANSBERGEN, G., & ROOD, J.J. VAN (1977) Effect of blood transfusions and HLA matching on renal transplantation in The Netherlands, Transpl. Proc. 9, 503-505

REHFELD, C.E. (1970) Definition of relationships in a closed beagle colony. Am. J. Vet. Res. 31, 723-732

RINGDEN, G., MOELLER, E., LUNDGREN, G. & GROTH, C.G. (1976) Role of MLC compatibility in intrafamiliar kidney transplantation. Transplantation 22, 9-17

ROOD, J.J. VAN, EERNISSE, J.G. & LEEUWEN, A. VAN (1958) Leukocyte antibodies in sera from pregnant woman. Nature 181, 1735-1736

ROOD, J.J. VAN (1962) Leucocyte grouping. A method and its application. Thesis Leiden University, Pasmans, Den Haag, the Netherlands

ROOD, J.J. VAN & LEEUWEN, A. VAN (1963) Leukocyte grouping. A method and its application. J. Clin. Invest. 42, 1382-1390

ROOD, J.J. VAN, LEEUWEN, A. VAN & BRUNING, J.W. (1967) The relevance of leucocyte antigens for allogeneic renal transplantation. J. Clin. Path. 20, 504-512

ROOD, J.J. VAN, BLUSSE VAN OUD ALBLAS, A. KEUNING, J.J., FREDERIKS, E., TERMIJTELEN, A., HOOFF, J.P. VAN, PENA, A.S. & LEEUWEN, A. VAN (1975) Histocompatibility genes and transplantation antigens. Transpl. Proc. 7, (suppl. 1) 25-30

ROOD, J.J. VAN, LEEUWEN, A. VAN, PERSYN, G.G., LANSBERGEN, G., GOULMY, E., TERMIJTELEN, A. & BRADLEY, B.A. (1977) HLA compatibility in clinical transplantation. Transpl. Proc. 9, 459-467

ROOD, J.J. VAN (1978) The relevance of HLA-D in renal transplantation. Proceedings 9th international course of transplantation and clinical immunology, Lyon 1977. Excerpta Medica, Amsterdam, in press

RUDOLPH, R.H. HERED, B., EPSTEIN, R.B. & THOMAS, E.D. (1969) Canine mixed leucocyte reactivity and transplantations antigens. Transplantation 8, 141-146

SACHS, J.A., FESTENSTEIN, H., TUFFNEL, V.A. & PARIS, A.M.I. (1977) Collaborative scheme for tissue typing and matching in renal transplantation. (IX.) Effect of HL-A, -B, and -D locus matching, pretransplant transfusion and other factors on 612 cadaver renal grafts. Transpl. Proc. 9, 483-486

SASPORTES, M., LEBRUN, A., RAPAPORT, F.T. & DAUSSET, J. (1972) Studies of skin allograft survival and mixed lymphocyte culture reactions in HL-A genotyped families. Transpl. Proc. 4, 209-218

SCANDIATRANSPLANT REPORT. HLA matching and kidney graft survival (1975) Lancet i, 240-242

SEGALL, M., BACH, F.H., BACH, M.L. HUSSEY, J.L. & UEHLING, D.T. (1975) Correlation of MLC stimulation and clinical course in kidney transplants. Transpl. Proc. 8, 41-43

SERRE, A. & CLOT, J. (1968) Les tests d'histocompatibilité chez le chien en vue de l'experimentation des greffes d'organes. Rev. Fr. Etud. Clin. Biol. 13, 1019-1024

SHEEHY, M.J., SONDEL, P.M., BACH, M.L., WANK, R. & BACH, F.H. (1975) HL-A LD typing: a rapid assay using primed lymphocytes. Science 188, 1308-1310

SHREFFLER, D.C. & DAVID, C.S. (1975) The H-2 major histocompatibility complex and the I immune response region: genetic variation, function and organization. Adv. Immunol. 20, 125-195

SILVERS, W.K., WILSON, D.B. & PALM, J. (1967) Typing and immunosuppression in rats. Transplantation 5, 1053-1056

SINGAL, D.P., MICKEY, M.R. & TERASAKI, P.I. (1969) Serotyping for homotransplantation. XXIII. Analysis of kidney transplants from parental versus sibling donors. Transplantation 7, 246-258

SINGH, R., VRIESENDORP, H.M., MECHELSE, K. & STEFANKO, S. (1977) Nerve allografts and histocompatibility in dogs. J. Neurosurg. 47, 737-743

SNELL, G.D. (1970) Immunologic enhancement. Surgery, Gynaecology and Obstetrics, 130, 1109-1119

SOLHEIM, B.G., FLATMARK, A., ENGER, E., JEWELL, J. & THORSBY, E. (1977) Influence of HLA-A, -B, -C and -D matching on the outcome of clinical kidney transplantation. Transpl. Proc. 9, 475-478

STARZL, T.E., MARCHIORO, T.L., HOLMES, J.H., HERMANN, G., BRITTAIN, R.S., STONINGTON, O.H., TALMAGE, D.W. & WEDDELL, W.R. (1964) Renal homografts in patients with major donor-recipient blood group incompatibilities. Surgery 55, 195-200

SUCIU-FOCA, N. & DAUSSET, J. (1975) Complex family patterns. Immunogenetics 2, 389-391

TEMPLETON, J.W. & THOMAS, E.D. (1971) Evidence for a major histocompatibility locus in the dog. Transplantation 11, 429-431

TERASAKI, P.I., KREISLER, M. & MICKEY, M.R. (1971) Presensitization and kidney transplant failures Postgraduate Medical Journal 47, 89-100

TERASAKI, P.I., PARK, M.S., OPELZ, G., SAITO, S. & MICKEY, M.R. (1977) The role of B-lymphocyte antigens in MLC stimulation. Transpl. Proc. 9, 469-473

THOMAS, F., MENDEZ-PICON, G., THOMAS, J. & LEE, H.M. (1977) Quantitation of pretransplantation immune responsiveness by in vitro T-cell testing. Transpl. Proc. 9, 49-53

THOMPSON, J.S., CORRY, R.J., LAWTON, R.L., BONNEY, W.W. & KALCKANIDES, G.J. (1977) Effect of prospective HLA haplotype matching in renal transplantation. Transpl. Proc. 9, (suppl) 205-207

THORSBY, E. (1974) The human major histocompatibility system. Transpl. Rev. 18, 51-129

TINBERGEN, W.J. (1971) Rat kidney transplantation. An evaluation of immunosuppression. Thesis - Leiden University. Waltman, Delft, the Netherlands

TWEEL, J.G. VAN DEN, VRIESENDORP, H.M., TERMIJTELEN, A., WESTBROEK, D.L., BACH, M.L. & ROOD, J.J. VAN (1974) Genetic aspects of canine mixed lymphocyte cultures. J. Exp. Med. 140, 825-835

UEHLING, D.T., HUSSEY, J.L., BELZER, F.O., KAHN, C. & BACH, F.H. (1977) MLC incompatibility index for donor-recipient matching. Transpl. Proc. 9, 103-105

VAIMAN, M., HAAG, J., ARNOUX, A. & NIZZA, P. (1973) The histocompatibility complex SL-A in the pig. Possible recombination between the regions governing MLR and serology respectively. Tissue antigens 3, 204-211

VRIESENDORP, H.M., ROTHENGATTER, C., BOS, E., WESTBROEK, D.L. & ROOD, J.J. VAN (1971) The production and evaluation of dog allolymphocytotoxins for donor selection in transplantation experiments. Transplantation 11, 440-445

VRIESENDORP, H.M. (1973) Major histocompatibility complex of the dog. Thesis, Erasmus University Rotterdam. Bronder Offset, Rotterdam, the Netherlands

VRIESENDORP, H.M., BIJNEN, A.B., ZUERCHER, C. & BEKKUM, D.W. VAN (1975[a]) Donor selection and bone marrow transplantation in dogs. In F. Kissmeyer-Nielsen (ed.) Histocompatibility testing p. 963-971

VRIESENDORP, H.M., ZUERCHER, C., BULL, R.W., LOS, W.R.T., MEERA KHAN, P., TWEEL, J.G. VAN DEN, ZWEIBAUM, A. & BEKKUM, D.W. VAN (1975[b]) Take and graft versus host reactions of allogenic bone marrow in tissue typed dogs. Transpl. Proc. 7, (suppl. I) 849-853

VRIESENDORP, H.M., BIJNEN, A.B., ZUERCHER, C. & BEKKUM, D.W. VAN (1976) Donor selection and bone marrow transplantation in dogs. Histocompatibility testing 1975, 963-971. Munksgaard, Copenhagen

VRIESENDORP, H.M., BIJNEN, A.B., WESTBROEK, D.L. & BEKKUM, D.W. VAN (1977[a]) Genetics and transplantation immunology of the DLA complex. Transpl. Proc. 9, 293-296

VRIESENDORP, H.M., GROSSE-WILDE, H. & DORFF, M.E. (1977[b]) The major histocompatibility complex of the dog. In: Götze, D. (ed) The major histocompatibility system in man and animals p. 129 -163 Springer Verlag, New York

VRIESENDORP, H.M., BIJNEN, A.B., KESSEL, A.C.M. VAN, OBERTOP, H. & WESTBROEK, D.L. (1978) Minor histocompatibility systems in dogs. Exp. Haemat. Today, Vol. II, Springer, New York, in press

WILLIAMS, R.M. & MOORE, M.J. (1977) Genetic recombination between serologically defined antigens and mixed lymphocyte reactivity in the rat major histocompatibility complex. Transpl. Proc. 9, 563-565

WESTBROEK, D.L., SILBERBUSCH, J., VRIESENDORP, H.M., URK, H. VAN, ROEMELING, H.W., SCHONHERR-SCHOLTES, Y. & VRIES, M.J. DE (1972) The influence of DL-A histocompatibility on the function and pathohistological changes in unmodified canine renal allografts. Transplantation 14, 582-589

WESTBROEK, D.L., VRIESENDORP, H.M., TWEEL, J.G. VAN DEN, GRUYL, J. DE & URK, H. VAN (1975) Influence of SD and LD matching on kidney allograft survival in unrelated mongrel dogs. Transpl. Proc. 3, 427-429

YUNIS, E.J. & AMOS, D.B. (1971) Three closely linked genetic systems relevant to transplantation. Proceedings of the National Academy of Sciences, United States of America 68, 3031-3035

ZWEIBAUM, A., ORIOL, R., FEINGOLD, N., DUSSAULX, E., ROUSSET, M. & CHEVALIER, G. (1974) Studies on canine secretory alloantigens (CSA). Tissue Antigens 4, 115-129

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