Nerve allografts and histocompatibility in dogs

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The histocompatibility requirements for successful frozen nerve allografts were studied in 46 dogs. Major canine histocompatibility (DLA) differences appeared to be of vital importance for nerve regeneration and function, as judged by histological and electromyographic performance 7 to 9 months after grafting. Minor histocompatibility differences did not appear to lead to rejection of the frozen nerve allografts. Graft irradiation did not improve the acceptability of frozen DLA-mismatched grafts. The effect of DLA matching was much more pronounced in allografts 7 cm long than in allografts 4 cm long. The results indicate the need for a bank of frozen human histocompatible (HLA) nerve allografts, and a study of the effect of partial or complete HLA matching on their survival.

KEY WORDS - nerve transplantation - histocompatibility - deep frozen nerve graft - nerve regeneration - irradiated nerve grafts - long nerve grafts

NERVE allografts have been performed in both experimental animals and human patients for more than a century. As early as 1870, Philipeaux and Vulpian demonstrated that nerve fibers can grow through a nerve graft, and in 1878 Albert reported a nerve allograft taken from an amputated limb to bridge a 3 cm-long defect of a median nerve. In more recent studies it was found that some nerve allografts up to 3 cm long could survive under various experimental conditions. Longer allografts have failed, almost without exception. In contrast, autografts have met with fair success since the good results of Mayo-Robson in 1917. With the recent progress in microsurgical techniques the results of autologous nerve cable grafts have become even more impressive.

In all probability the difference in end results between allogeneic and autologous tissues lies in the immunological rejection of the former. A further investigation of the less successful nerve allografts is, however, indicated, since autologous nerves are not always available in sufficient lengths, and the normal neurolemmal pattern of the parent nerve is never replaced in this situation. Moreover, neurological deficits are sometimes produced by the removal of the grafts from their original anatomical location. To ensure a successful allograft the host response against the allogeneic nerve tissue must be prevented. Immunosuppressive agents, Wallerian degeneration, and irradiation of the graft have been used to achieve this goal. Despite promising results in early experiments, disappointing clinical
RESULTS were obtained with deep frozen irradiated allografts.\textsuperscript{7,17,18} In the last decade, studies of transplantation immunology have elucidated the genetic control of immune reactivity against allogeneic tissues in a number of mammalian species. One polymorphic chromosomal area with a major influence on allograft survival has been identified in each species investigated so far. This area has been labeled the major histocompatibility complex (MHC). Matching for structures controlled by the MHC is beneficial for the survival of allogeneic tissues of diverse histological types.\textsuperscript{25} The aim of the present study was to identify the influence of the MHC on the survival of nerve allografts of different lengths in dogs. The effect of pretransplant graft irradiation was also evaluated.

Materials and Methods

Experimental Animals

We used 46 beagles, divided into eight groups as shown in Table 1.

Tissue Typing

The dogs were typed for serologically defined leukocyte antigens belonging to the canine MHC, which is called “DLA,” by a battery of approximately 80 antisera. The antisera, their analysis, and the one-stage microcytotoxicity test have been described previously.\textsuperscript{20,23,27} Littermate dogs were grouped in pairs with no, one, or two DLA haplotype differences. The term “haplotype” is used as defined in humans by Ceppelini, \textit{et al.}\textsuperscript{2}

Nerve Grafting

Peroneal nerves, 4 or 7 cm in length, were removed from all 45 dogs under general anesthesia and under sterile conditions. Nerves were preserved in tissue culture Medium 96 in sterile, sealed bags at a minimum constant temperature of $-70^\circ$C for a period of 4 to 6 weeks. In one set of experiments 23 of these frozen grafts were irradiated with 1.66 Mrad for 10 seconds at the temperature of dry ice in a van de Graaff generator.\textsuperscript{*} The transplantation was carried out orthotopically using standard microsurgical techniques with $\times 16$ magnification, and 10–0 atraumatic monofilament nylon for suturing.

Evaluation of Grafts

The neurological examination of a dog is not an objective procedure and can lead to biased results. Therefore electromyographic (EMG) and histological techniques were used to evaluate the results obtained 7 to 9 months after transplantation. This period is required to allow the growing axons to reach the denervated muscle through the transplanted and autologous nerve sheath.

Electromyographic Examination. Grafts were reexposed under general anesthesia without using muscular relaxants. The nerve was stimulated directly with bipolar platinum electrodes. Four electrodes were placed at

\textsuperscript{*}Van de Graaff generator manufactured by High Voltage Engineering Co., Amersfoort, The Netherlands.

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\begin{table}
\centering
\begin{tabular}{lllll}
\hline
Group No. & No. of Recipients & No. of DLA Haplotype Differences & Graft Irradiation & Nerve Length (cm) \\
\hline
1 & 5 & 0 & + & 4 \\
2 & 5 & 1 & + & 4 \\
3 & 6 & 2 & + & 4 \\
4 & 6 & unrelated mismatched & + & 4 \\
5 & 6 & 0 & - & 4 \\
6 & 6 & unrelated mismatched & - & 4 \\
7 & 6 & 0 & - & 7 \\
8 & 6 & unrelated mismatched & - & 7 \\
\hline
\end{tabular}
\caption{Experimental groups used in study}
\end{table}

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\textsuperscript{*}Number of DLA haplotype differences between donor and recipient.
fixed distances of 2 cm along the nerve. The first electrode was placed proximal to the graft and the last electrode distal to the graft. The compound action potential of the anterior tibial muscle was recorded through a concentric needle electrode. The nerve was stimulated supramaximally with a square wave of 0.3 msec duration. The temperature of the nerve ranged between 27° and 30° C. The maximum conduction velocity of the graft was estimated from latency of the proximal and distal stimulating point to the anterior tibial muscle and the conduction distance. In the 20 control dogs investigated that did not undergo grafting, the maximum conduction velocity of the peroneal major nerves ranged from 30 to 50 m/sec. Results were grouped by conduction time into four EMG grades as follows:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Conduction Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal (40 m/sec)</td>
</tr>
<tr>
<td>II</td>
<td>20 to 30 m/sec</td>
</tr>
<tr>
<td>III</td>
<td>15 to 19 m/sec</td>
</tr>
<tr>
<td>IV</td>
<td>No response</td>
</tr>
</tbody>
</table>

**Histological Examination.** The transplanted nerve was removed with 1 cm of host nerve at each end of the graft. Longitudinal sections at proximal and distal transplant suture sites, and transverse sections at the proximal and distal segments of the host nerve were made. Appropriate stains were used to visualize axonal regeneration, myelinization, and inflammatory reactions. The results were grouped into four histological grades as follows:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Good, isomorphic regeneration with developing myelinization. No fibrosis or inflammatory infiltrate (Fig. 1).</td>
</tr>
<tr>
<td>II</td>
<td>Good, slight anisomorphic regeneration with developing myelinization, slight fibrosis, no inflammatory infiltrate.</td>
</tr>
<tr>
<td>III</td>
<td>Poor regeneration with peripheral anisomorphy. No myelinization. Aberrant fibrosis.</td>
</tr>
<tr>
<td>IV</td>
<td>Poor regeneration with marked perineural and intraneural anisomorphy. Extensive fibrosis and inflammatory infiltrate (Fig. 2).</td>
</tr>
</tbody>
</table>

A more detailed and illustrated description of these grades has been reported previously. 16,19

**Results**

**Histocompatibility and Nerve Allograft Survival**

The influence of matching for the major histocompatibility complex on the performance of nerve allografts 7 to 9 months after grafting can be seen in Table 2. An almost normal EMG and histological response is observed in the DLA-identical donor-recipient combinations (experimental Group 1). Groups 2, 3, and 4 represent a gradual
TABLE 2

Influence of matching for major histocompatibility complex on nerve allografts in dogs
7 to 9 months after transplant*

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Donor-Recipient Relationship</th>
<th>No. of Dogs in Electromyographic Grade</th>
<th>No. of Dogs in Histological Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I II III IV</td>
<td>I II III IV</td>
</tr>
<tr>
<td>1</td>
<td>DLA-identical</td>
<td>3 2 -- --</td>
<td>4 1 -- --</td>
</tr>
<tr>
<td>2</td>
<td>one DLA haplotype different</td>
<td>1 3 -- 1</td>
<td>-- 5 -- --</td>
</tr>
<tr>
<td>3</td>
<td>two DLA haplotypes different</td>
<td>-- 1 2 3</td>
<td>-- 3 3</td>
</tr>
<tr>
<td>4</td>
<td>unrelated, DLA-mismatched</td>
<td>-- -- 3 3</td>
<td>-- 3 3</td>
</tr>
</tbody>
</table>

*All grafts were 4 cm long, cryopreserved, and irradiated.

decrease in matching (or increase in mismatching). A positive correlation between the DLA match and the performance and histology of the nerve is observed in all groups. The only partially DLA-mismatched group, Group 2, is doing better than the completely mismatched Groups 3 and 4.

Graft Irradiation and Nerve Allograft Survival

Irradiated and non-irradiated frozen nerve allografts are compared in Table 3. As shown by the data in Table 2, DLA compatibility is an important parameter in the evaluation of the performance and histology of a nerve graft. It is evident that in DLA-matched and in mismatched grafts the survival qualities of the nerves are not improved upon by the irradiation of the frozen graft before irradiation.

Nerve Length and Allograft Survival

Even with grafts 7 cm long a good regeneration and functional survival was found if the donor was identical in DLA to the recipient (Table 4). In contrast, in none of the DLA-mismatched grafts of 7 cm length was regeneration or functional survival noted. However, in some grafts 4 cm in length incomplete regeneration was noticed in DLA-mismatched combinations. Therefore, histocompatibility matching appears to be of even greater importance in the survival of longer nerve allografts.

Discussion

This report shows that frozen DLA-identical nerve allografts can regenerate and function longer than 7 to 9 months after transplantation as judged by EMG or histo-

Fig. 2. Photomicrographs from dogs in histological Grade IV. *Left:* Site of nerve suture showing an anisomorphic neuroma. There is considerable proliferation of connective tissue and aberrant fibers. Van Gieson, × 167. *Right:* Distal nerve segment showing diffuse inflammatory reaction in epineurium and perineurium. H & E, × 167.
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TABLE 3
Influence of graft irradiation on survival of frozen nerve allografts 7 to 9 months after transplant

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Donor-Recipient Relationship</th>
<th>No. of Dogs in Electromyographic Grade</th>
<th>No. of Dogs in Histological Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1</td>
<td>DLA-identical, irradiated</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>DLA-identical</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>DLA-mismatched, unrelated, irradiated</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>DLA-mismatched, unrelated</td>
<td>--</td>
<td>4</td>
</tr>
</tbody>
</table>

 logical criteria. In view of the experience with histocompatible allografts in dogs or other species, one can assume with some confidence that these grafts, if left undisturbed, would have continued to function in a normal way. The very long survival for each single, frozen, DLA-identical nerve allograft performed is different from the prolonged, but limited survival of transplants of other vital tissues of a DLA-identical donor. This difference might be due to 1) a lack of effect of minor histocompatibility structures on nerve allografts; 2) the disappearance of minor histocompatibility structures on nerve allografts by the storage at -70°C or irradiation of the graft with 1.66 Mrad. Verhoog and van Bekkum concluded that major histocompatibility structures on nerve allografts in rats disappeared under these treatments. The difference between their results and ours might be explained by the following factors: 1) the 2-week observation period used by Verhoog and van Bekkum, whereby a rejection process occurring later might have been missed; 2) the 0.5-mm rat grafts used, which were too small to show the effect of major histocompatibility structures; and 3) the major histocompatibility difference between the rat strains used, which might be comparable to the one DLA haplotype difference used in this study. The latter grafts appear to do almost as well as DLA-identical grafts.

In contrast to the absence of any effect of minor histocompatibility structures is the short survival of the grafts with two different DLA haplotypes, or the unrelated DLA-mismatched grafts. These grafts did not survive the 7- to 9-month observation period. This demonstrates that major histocompatibility structures are present on nerve allografts after storage at -70°C and/or irradiation of the graft with 1.66 Mrad.

TABLE 4
Influence of nerve lengths on allograft survival 7 to 9 months after transplant

<table>
<thead>
<tr>
<th>Group No</th>
<th>Donor-Recipient Relationship</th>
<th>Nerve Length (cm)</th>
<th>No. of Dogs in Electromyographic Grade</th>
<th>No. of Dogs in Histological Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>7</td>
<td>DLA-identical</td>
<td>7</td>
<td>5</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>DLA-identical</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>DLA-mismatched, unrelated</td>
<td>7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>DLA-mismatched, unrelated</td>
<td>4</td>
<td>--</td>
<td>4</td>
</tr>
</tbody>
</table>
degree but not sufficiently for long-term protection in severely DLA-mismatched donor-recipient combinations.

There might also be a qualitative rather than a quantitative loss of DLA structures in deep frozen and thawed nerve allografts. If this is the case, it would offer a simplified situation and allow for an easier identification of the true histocompatibility determinants among the various structures controlled by the DLA region. The influence of DLA matching was more pronounced in longer grafts (Table 4), which is in accordance with earlier results in man where regeneration was found in only a few nerve allografts of 3 cm or shorter. Donor-recipient pairs differing for one DLA haplotype were not tested using 7-cm long nerve allografts. It remains to be seen whether the good regeneration and function found in this donor-recipient combination with the shorter (4 cm long) nerve allografts, can also be demonstrated in the 7-cm long nerve allografts, which appear to have stricter histocompatibility requirements.

An important difference between DLA in related and unrelated donor-recipient pairs, has been demonstrated for kidney allografts and for bone-marrow grafts. The beneficial effect seen with identical DLA on allograft survival was absent or less pronounced in the unrelated host-donor combinations. An explanation for this difference is given elsewhere.

A good histocompatibility match appears to be essential for a prolonged good functional survival and longer nerve allografts. However, the histocompatibility requirements for survival of small frozen nerve allografts appear to be less strict in comparison to other organ grafts and a partial, incomplete matching for human histocompatibility (HLA) structures might be sufficient for an effective nerve allograft survival in unrelated human donor-recipient pairs.

When these data are extrapolated to the possible requirements for successful nerve allografting in man, it becomes evident that an analysis of the effect of HLA matching on nerve allograft survival might be rewarding. The institution of a bank of frozen nerves of as many different lengths and diameters, and of as many different HLA types as possible would be needed for such an investigation. Animal studies, such as the present one, have shown that this is a realistic goal that might be of benefit for human patients in need of a nerve allograft.

References
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