The host environment determines strain-specific differences in the timing of skeletal muscle regeneration: cross-transplantation studies between SJL/J and BALB/c mice

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ABSTRACT

The difference in the timing of the regeneration process of skeletal muscle between SJL/J and BALB/c mice was investigated using grafts of whole skeletal muscle (both autografts and allografts). Histological, autoradiographic and immunohistochemical techniques were used in the investigation. Infiltration of leucocytes into autografts, numbers of desmin-positive myogenic cells and myotube formation were all more advanced in the SJL/J compared with BALB/c mice. Furthermore, autoradiographic evidence showed that myoblasts in the SJL/J autografts were synthesising DNA 12 h earlier than myoblasts in BALB/c autografts. In allografts, where SJL/J host mice received BALB/c grafts, and vice versa, leucocyte infiltration and myotube formation occurred earlier in the BALB/c muscles grafted into SJL/J hosts, than in the reverse situation with BALB/c hosts. The results show that, at least for whole muscle grafts, it is the host environment which determines the speed and outcome of the regenerative process.

Key words: Muscle autografts and allografts; leucocytes; myotubes; desmin; autoradiography.

INTRODUCTION

Studies investigating the regeneration of muscle after crush injury (Grounds & McGeachie, 1989; Mitchell et al. 1992) and in minced isografts (Grounds, 1987; Grounds & McGeachie, 1990) in SJL/J and BALB/c mice have reported a superior and more extensive regeneration in SJL/J mice. This enhanced regeneration in SJL/J mice includes an earlier onset of muscle precursor cell replication (6 h earlier in crush injuries, 18 h in minced isografts), a 2-fold increase in inflammatory cells at 3 d in crushed muscles, and earlier myotube formation associated with more frequent and larger myotubes. Tissue culture studies of muscle from the 2 strains support these in vivo differences as activation of the skeletal muscle specific genes, MyoD and myogenin, is earlier (Maley et al. 1994) and myotube formation much more effective (Maley et al. 1995) in primary cultures of adult SJL/J, compared with BALB/c muscle. In all of these situations (crush injury, mincing, tissue culture) there is massive damage and disruption of the normal cellular relationships and muscle architecture.

In contrast, there is relatively little damage to the myofibre structure in whole muscle grafts and new muscle formation is usually excellent as it occurs within the scaffold of a relatively intact, persisting external lamina of the necrotic myofibres (Vracko & Benditt, 1972; Hansen-Smith & Carlson, 1979). Previous studies of the regeneration of whole skeletal muscle grafts have determined the time course of this process in BALB/c mice (Roberts et al. 1989; Roberts & McGeachie, 1990). It is of interest to determine whether strain-specific differences in muscle regeneration might also be apparent in whole muscle grafts. This was examined by analysis of autografted extensor digitorum longus muscles (EDL), sampled at 2–14 d after transplantation, in SJL/J and BALB/c mice.

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determine whether it is a characteristic of the muscle of the SJL/J mice which allows it to regenerate faster and more extensively than BALB/c mice, or whether this process is dependent upon exogenous host influences (outside the muscle). EDL muscles were also cross-transplanted (allografted) between SJL/J and BALB/c mice and examined histologically. In addition, immunohistochemical analyses were done on another set of grafts to detect desmin which is an early marker for myogenic precursor cells and myotubes (Kaufman & Foster, 1988; Allen et al. 1991).

**Materials and Methods**

**Animals and surgical procedures**

A total of 31 young mature male BALB/c and SJL/J mice (aged 6–8 wk at the time of surgery) were used in this study. Nine BALB/c and 10 SJL/J mice received autografts (38 autografts in total), whilst allografts were performed between 6 BALB/c and 6 SJL/J mice (24 allografts in total). The transplantation procedure has been described in detail elsewhere (Roberts et al. 1989; Roberts & McGeachie, 1990, 1995). Briefly, the mice were anaesthetised with a gaseous mixture of halothane/N2O/O2 before removing the EDL muscles from both the hindlimbs of each mouse and relocating the EDLs over the tibialis anterior muscles (TA) of the same legs from which they were removed. Each EDL was sutured proximally to the distal tendon of the quadriceps femoris muscle, and distally to the quadriceps femoris muscle, and distally to the distal tendon of the TA, and the skin closed (Roberts & McGeachie, 1995). The allografting procedure involved removing the EDL muscles from both hind limbs of BALB/c and SJL/J mice and cross-transplanting the muscles onto the TA of mice of the opposite strain, i.e. the EDL muscles from BALB/c mice were transplanted into SJL/J mice and vice versa.

**Sample removal and histological analysis**

Each mouse was anaesthetised with a lethal dose of pentobarbitone sodium (80 mg/kg) before being killed by cervical dislocation. Samples were taken at: 2, 3, 4, 5, 6, 7, and 14 d after transplantation. The transplanted EDL and part of the underlying TA muscle were dissected free, cut transversely in half and immersed overnight in full-strength fixative (0.1 M phosphate buffered 10% formalin, pH 7.2) at 4°C. The tissues were placed into a Lynx automatic tissue processor, washed in 0.1 M phosphate buffer, and then dehydrated through a series of graded ethanol before being infiltrated and embedded in Araldite. Transverse sections (1 µm) were cut from the central area of each transplant and placed on glass slides. Sections from each mouse were taken for routine light microscopic histology. The sections for routine light microscopic observation were stained with haematoxylin and cosin (H&E).

All samples were coded, then analysed 'blindly'. Sections were examined at low power through to high power (under ×100 oil immersion) and analysed histologically, specifically for the infiltration of leucocytes and the presence of myotubes from the periphery of the transplant towards the centre. Myotubes were defined as cells with a diameter greater than 15 µm, an open-faced central nucleus, and condensed cytoplasm. Four sections per transplant were analysed and an eyepiece graticule inserted into the microscope was used to measure infiltration distances.

**Autoradiography**

At 36 h after transplantation, 1 autografted SJL/J mouse received a single intraperitoneal injection of tritiated thymidine ([3H]Tdr, 1 µCi/g of body weight, specific activity 5 Ci/mmol, Amersham, Australia) but was not killed until 14 d after the grafts were inserted. This was to allow time for muscle precursor cells which had been replicating (synthesising DNA) at the time of [3H]Tdr injection (at 36 h) to fuse and form myotubes, when the labelled nucleus can be readily identified as a myotube nucleus. Tissues for autoradiographic analysis were removed and post-fixed as described above, but were block-stained in 1% paraphenylenediamine (in 70% ethanol) for 1 h prior to the ethanol dehydration stage to obviate tissue staining following autoradiographic processing (Dilley & McGeachie, 1983). Sections were coated with NTB-2 autoradiographic dipping film and exposed in light-tight boxes for 2 wk, then developed in Kodak D19 and fixed in acid-hardener fixer, washed and dried for autoradiographic analysis. Postmitotic samples, in transplants which had been allowed to regenerate for 14 d, were examined for autoradiographically labelled myotube nuclei on a light microscope with a ×100 oil immersion lens. A myotube nucleus was not considered to be labelled unless it had a minimum of 3 autoradiographic grains present in the emulsion overlying it (Roberts et al. 1989).

**Desmin immunohistochemistry**

**Grafts.** EDL autografts (2 in each mouse) were inserted into 3 mice: 2 SJL/J and 1 BALB/c. EDL allografts (2 in each mouse) were also inserted into 2
Table 1. Numbers of leucocytes and myotubes present in grafts at various times after transplantation. Myotubes were not seen in grafts removed before 72 h

<table>
<thead>
<tr>
<th>Time grafts removed (d) after insertion</th>
<th>Autografts</th>
<th>Autografts</th>
<th>Allografts</th>
<th>Allografts</th>
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<tr>
<td></td>
<td>SJL/J donor</td>
<td>BALB/c donor</td>
<td>SJL/J host</td>
<td>BALB/c host</td>
</tr>
<tr>
<td>2</td>
<td>*</td>
<td>—</td>
<td>No grafts at 2 d</td>
<td>No grafts at 2 d</td>
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<td>3</td>
<td>** +</td>
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<td>*** + + +</td>
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<tr>
<td>6</td>
<td>No grafts at 6 d</td>
<td>No grafts at 6 d</td>
<td>Immune reaction</td>
<td>Immune reaction</td>
</tr>
<tr>
<td>7</td>
<td>Comp  Comp</td>
<td>*** + + +</td>
<td>Immune reaction</td>
<td>Immune reaction</td>
</tr>
</tbody>
</table>

Leucocyte infiltration into the grafts, as measured from the periphery: —, no cellular infiltration; * cellular infiltration; up to 100 µm; ** cellular infiltration, up to 200 µm; *** cellular infiltration, up to 300 µm; **** cellular infiltration, up to 400 µm; comp, cellular infiltration to centre of transplant.

Myotubes: —, no myotubes; +, 1–10 myotubes; ++, 11–20 myotubes; ++++, 21–40 myotubes; ++++, > 40 myotubes; comp, myotubes to centre of transplant. Myotubes were viewed in transverse section.

Fig. 1. Autografts at 2 d showing the interface between the EDL grafts and the underlying TA (at the bottom, but not shown). Necrotic muscle fibres are evident. Leucocytes have infiltrated ~100 µm into the SJL/J grafts (a) but not into the BALB/c grafts (b). Both photomicrographs are the same magnification.

mice: 2 SJL/J grafts into a BALB/c host and 2 BALB/c grafts into a SJL/J host. One of the SJL/J mice with 2 autografts was killed (as above) at 2 d after grafting, to see if myogenesis (desmin staining) had commenced at that early time. The other 4 autografts and 4 allografts were removed at 3 d after grafting.

**Tissue preparation.** At the time of removal the hind limbs were placed in 4% paraformaldehyde fixative for 15 h and stored in 70% ethanol overnight. The TA (with the EDL graft attached) was dissected free from the underlying limb and cut transversely midway between the tendon sutures. The specimens were dehydrated through ascending ethanol concentrations and embedded in paraffin wax. Transverse sections were cut at 6 µm, floated onto silane-coated slides and placed in an oven at 60 °C. Antigen retrieval was carried out in citrate buffer at 60 °C (modified from Cattoretti & Suurmeijer 1995; Shi et al. 1995) before the desmin antibody was applied.

**Antibodies.** A polyclonal rabbit desmin antibody (Biogenex) was used in conjunction with biotin–avidin and a DAB substrate (Evers & Uyling, 1994).

**Analysis of desmin stained sections.** All the 10 EDL grafts were analysed ‘blindly’ (as were the light microscopic sections and autoradiographs) and, after the data were recorded, the code was broken to identify the animal from which the grafts were taken. A simple ++ scoring system was used: the lowest numbers of desmin-positive cells scoring +, and the highest numbers, ++++. This system is detailed in the Results section.
Fig. 2. Autografts at 3, 5 and 7 d, showing the interface between the EDL grafts and the underlying TA (at the bottom the figures, but not shown in most panels). This plate of photomicrographs compares the cellular changes with time between SJL/J mice (on the left) and BALB/c mice (on the right). All photomicrographs are the same magnification. (a) 3 d SJL/J autografts consist mainly of pale staining
RESULTS

The histological appearance of the grafts followed the pattern of regeneration described in detail by Roberts et al. (1989) and Roberts & McGeachie (1990). There was a concentric pattern of regeneration from the periphery of the transplants towards the centre, the regeneration being preceded by leucocyte infiltration. However, the timing of regeneration, as measured by both leucocyte infiltration and the appearance of myotubes within the transplants differed between the autografts in SJL/J and BALB/c mice, and in the cross-transplanted grafts in the different hosts (Table 1).

Autografts

In 2 d grafts from SJL/J mice, leucocytes had infiltrated the transplant to a depth of 100 µm from the periphery (Fig. 1a), whereas in 2 d grafts from BALB/c mice, no leucocyte infiltration was evident (Fig. 1b).

By 3 d, leucocytes had infiltrated SJL/J autografts up to a depth of 200 µm (Fig. 2a) and very occasional myotubes were present in the periphery of the grafts. In BALB/c autografts, leucocytes had infiltrated to a depth of 100 µm (Fig. 2b), but there was no evidence of small myotubes. By 5 d in SJL/J autografts, there was a well defined band of myotubes in the 100–200 µm layer adjacent to the TA (Fig. 2c), and in the BALB/c grafts far fewer myotubes were confined to a 50–100 µm layer (Fig. 2d).

The pattern of cellular events in BALB/c autografts was approximately 24 h behind that of the autografts in SJL/J mice, in terms of both leucocyte infiltration and myotube formation. These differences in the patterns of muscle regeneration were still evident until the last time sampled (7 d), when the SJL/J grafts were filled to the centre with myotubes (Fig. 2e). This compares with 7 d BALB/c autografts where there was still a central core of necrotic muscle fibres (Fig. 2f), and leucocytes had not infiltrated the centre of the graft (Table 1).

Allografts

When EDL muscles from BALB/c mice were allografted into SJL/J host mice, myotubes were first seen 3 d after transplantation, with leucocytes infiltrating the transplants to a depth of 200 µm (Fig. 3a). By contrast, examination of the 3 d EDL allografts of SJL/J muscle transplanted into BALB/c hosts detected no myotubes, although leucocytes were observed in the periphery of the transplants to a depth of 100 µm (Fig. 3b).

By 4 d after grafting, BALB/c allografts in the SJL/J host showed a heavy phagocytic infiltration up to a depth of 300 µm, with many myotubes present, whereas SJL/J allografts in the BALB/c host showed few myotubes and leucocyte infiltration to a depth of only 200 µm. At 5 d, allografts in both the SJL/J and BALB/c hosts had myotubes present (Fig. 3c, d), but with many more present in allografts in SJL/J hosts. Both sets of grafts removed from SJL/J and BALB/c hosts still contained a central core of necrotic myofibres, which had not been reached by infiltrating phagocytes. More leucocytes and myotubes were seen in the allografts in both hosts (BALB/c and SJL/J) than in any autografts at 5 d (see Discussion).

At 6 d, leucocytes had infiltrated completely to the centre of the necrotic core in allografts in the SJL/J host and no necrotic myofibres remained. In allografts in the BALB/c host, a central core of necrotic myofibres was still present which had not been reached by infiltrating leucocytes. These data from 6 d allografts, together with samples of 7 d allografts, were not included in the analysis because it was considered that an immune response had become established.

Autoradiography

It has been shown (Roberts et al. 1989; Roberts & McGeachie, 1990) that the earliest DNA synthesis (as identified by autoradiographic techniques) in whole EDL muscle grafts in BALB/c mice is 48 h post-transplantation. The 2 autografts examined here, taken from a single SJL/J mouse, were to determine whether the onset of DNA synthesis might occur earlier, at 36 h after transplantation, in this strain of mouse. In grafts examined at 14 d, 4 labelled myotubes were seen (1 is shown in Fig. 4) in a single transplant (and these had between 3 and 4 autoradiographic grains over each nucleus). In addition, 4 peripheral nuclei of immature muscle fibres (presumably satellite cells) were labelled with between 3 and 6 auto-

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*References*

- Roberts et al. 1989
- Roberts & McGeachie 1990
- Roberts et al. 1989
- Roberts & McGeachie 1990
Fig. 3. Allografts at 3 d and 5 d, showing the interface between the EDL grafts and the underlying TA (not shown). Photomicrographs a and b are at the same magnification as in Figure 2, and c and d are the same magnification as in Figure 1. In this Figure 3, the cellular changes with time are compared between SJL/J host mice (on the left) and BALB/c host mice (on the right). (a) BALB/c allografts into SJL/J hosts at 3 d have leucocytes invading the graft for some 200 µm. This graft resembles the SJL/J autografts at 3 d (Fig. 2a). (b) In comparison with a, SJL/J allografts into BALB/c hosts were much more quiescent with few leucocytes to be seen. This graft resembles the BALB/c autografts at 3 d (Fig. 2b). (c) At 5 d, BALB/c allografts into SJL/J hosts had an obvious infiltration of leucocytes and contained many myotubes. The latter were more numerous and more mature than in SJL/J grafts into BALB/c hosts (panel d). (d) At 5 d, SJL/J allografts into BALB/c hosts also had a leukocytic infiltration, but less so than in BALB/c grafts into SJL/J hosts (panel c), and some myotubes had developed.

Radiographic grains over their nuclei. This indicated that in the SJL/J autografts, DNA synthesis in presumptive myoblasts had been occurring at the time of \[^{3}H\]TdR injection (36 h after transplantation), which is at least 12 h earlier than in equivalent BALB/c grafts reported in a similar study (Roberts & McGeachie, 1990). This more rapid onset of myoblast replication correlates with the earlier infiltration of leucocytes, earlier detection of desmin-positive cells and earlier myotube formation seen in SJL/J autografts.

**Desmin immunohistochemistry**

**General comments.** Desmin staining was initially confined to the periphery of the EDL grafts, particularly at the TA interface. The density of desmin-positive cells and the location of these stained cells within the EDL varied with the time after grafting and the strain of the host mouse. The assessment of desmin stained cells was based on the numbers of nuclei surrounded by desmin stained cytoplasm (Fig. 5). Within a cluster of cells the maximal density of
Fig. 4. (a) Myotubes in an SJL/J autograft removed 14 d after grafting. (b) Same field as in a but focused on 3 autoradiographic grains in the emulsion overlying a labelled myotube nucleus, in the centre of the field. The animal from which this graft was sampled was injected with [H]TdR at 36 h after the graft was inserted. Therefore this labelled myotube nucleus was the progeny of a myogenic precursor cell labelled at the time of injection.

Fig. 5. Desmin staining (dark brown) in the cytoplasm of myoblasts in a BALB/c EDL allografted into a SJL/J host, sampled 3 d after grafting. The nuclei are counterstained blue/grey. This transverse section of the EDL was taken at the interface with the underlying TA muscle (not shown).

desmin-positive cells was 10% and this level (8–10%) was scored as 4+. The minimal level, scored as 1+, represented 1% of cells being stained; 2+ represented some 2–5% staining and 3+ was 6–8% of cells in a cluster being surrounded by desmin-positive cytoplasm.

Autografts. SJL/J autografts at 2 d had very few desmin-positive cells, those present were located in the peripheral 50 µm of the EDL graft adjacent to the TA (Table 2). By 3 d in SJL/J grafts, there were much heavier concentrations of desmin-positive cells and these extended some 200 µm into the EDL (Table 2).
Table 2. Distribution and comparative numbers of desmin stained cells in EDL grafts

<table>
<thead>
<tr>
<th>Mouse number, side</th>
<th>Days after grafting</th>
<th>Autografts: mouse strain</th>
<th>Allografts: mouse strain donor: host</th>
<th>Maximum distance of desmin-positive cells from TA (in µm)*</th>
<th>Score of desmin staining (+ ... + + + +)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 L</td>
<td>2</td>
<td>SJL/J</td>
<td>—</td>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>1 R</td>
<td>2</td>
<td>SJL/J</td>
<td>—</td>
<td>50</td>
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<td>2 L</td>
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<td>SJL/J</td>
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<td>2 R</td>
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<td>SJL/J</td>
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<tr>
<td>3 L</td>
<td>3</td>
<td>BALB/c</td>
<td>—</td>
<td>150</td>
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<td>3 R</td>
<td>3</td>
<td>BALB/c</td>
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<td>4 L</td>
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<td>SJL/J: BALB/c</td>
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<td>4 R</td>
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<td>SJL/J: BALB/c</td>
<td>100</td>
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<tr>
<td>5 L</td>
<td>3</td>
<td>BALB/c: SJL/J</td>
<td>250</td>
<td>+ + + +</td>
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<tr>
<td>5 R</td>
<td>3</td>
<td>BALB/c: SJL/J</td>
<td>250</td>
<td>+ + + +</td>
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</tbody>
</table>

Within a cluster of cells the maximal density of desmin-positive cells was 10%; this level (8–10%) was scored as + + + +. The minimal level was 1% and was scored as + ; ++ represented 2–5% staining and + + + + was 6–8% of cells in a cluster being desmin-positive.

* Desmin staining was much more pronounced in the grafts at the interface with the underlying TA. This area was sampled because there was only minimal staining in the subcutaneous region of the grafts at the times sampled.

The BALB/c grafts at 3 d had noticeably fewer desmin-positive cells and these penetrated some 100–150 µm into the graft (Table 2).

**Allografts.** BALB/c grafts transplanted into SJL/J hosts had a staining density similar to the SJL/J autografts. In both of the grafts examined the desmin-positive cells were seen some 250 µm into the EDL (Table 2). By contrast, SJL/J muscles grafted into BALB/c hosts had desmin-staining densities similar to BALB/c autografts, with stained cells penetrating some 100 µm into the EDL graft (Table 2).

In summary, the density and pattern of desmin staining (myogenic cell activity) in all EDL grafts were determined by the host environment: the SJL/J host strain produced vigorous myogenesis in both autografts and allograft, and vice versa in the BALB/c host strain.

**DISCUSSION**

**Timing of regeneration in autografts of the 2 strains**

This study was designed to investigate whether autografts of whole intact skeletal muscle regenerate faster and more extensively in SJL/J compared with BALB/c mice and, if so, to determine whether such differences are due to characteristics inherent in the grafted muscle, or to exogenous host influences. Previous studies have shown much better new muscle formation in SJL/J mice after crush injury, mincing and in tissue culture (see Introduction) and faster and more effective regeneration was also apparent in the SJL/J mice in the present study. The autoradiographic data show that myoblasts in the SJL/J autografts were synthesising DNA by 36 h, which is at least 12 h earlier than in BALB/c autografts where the onset of replication was at 48 h (Roberts et al. 1989; Roberts & McGeachie, 1990). Desmin-positive myoblasts were seen in very low numbers at the periphery in SJL/J autografts removed at 2 d. (A 2 d autograft was not done in a corresponding BALB/c mouse because previous experience had revealed that there is no activity in such grafts.) By 3 d, desmin-positive cells were seen in abundance in SJL/J autografts, and whilst some were also present in BALB/c grafts they were markedly fewer. Additionally, greater numbers of myotubes were present in the SJL/J than in BALB/c autografts, although this would be expected as the onset of myotube formation was 1 d earlier (at 3 d) in SJL/J autografts. The more rapid activation, replication and fusion of myoblasts in SJL/J autografts was associated with an earlier appearance of leucocytes and more rapid phagocytosis of necrotic tissue in the SJL/J autografts.

**Myotube formation**

Although the timing of the regenerative response was faster in the SJL/J autografts, the efficiency of myotube formation appeared similar in the whole muscle grafts in both strains. This contrasts with a striking difference in myotube formation seen between the 2 strains in situations where there has been major tissue damage and severe disruption to myofibre architecture. For example, myotube formation in grafts of minced muscle (Grounds, 1987; Grounds &
McGeachie, 1990) and in response to crush-injury (Grounds & McGeachie, 1989; Mitchell et al. 1992) is far more effective in SJL/J muscles than in BALB/c mice. This is also seen under tissue culture conditions (Maley et al. 1994, 1995) where more frequent and larger myotubes were seen in primary muscle cultures from SJL/J compared with BALB/c mice. This difference was very marked when myoblasts from the 2 strains were grown on gelatin (collagen 1) rather than on Matrigel, as myotubes in BALB/c cultures were sparse and very stunted (often only binucleated) compared with the many large myotubes in SJL/J cultures. The composition of Matrigel resembles that of the external lamina which is in intimate contact with satellite cells located on the surface of myofibres. Myotube formation was greatly enhanced in BALB/c cultures grown on Matrigel (Maley et al. 1995) and this resembles the situation in whole muscle grafts where excellent myotube formation occurs within the confines of the essentially intact, surviving external lamina of the necrotic myofibre (Vracko & Benditt, 1972; Hansen-Smith & Carlson, 1979). The fact that myotube formation seems equally effective (although slightly faster in SJL/J hosts) in whole muscle grafts of both strains strongly supports the idea that the presence of appropriate extracellular matrix molecules determines the efficiency of myoblast fusion.

Cross transplantation of grafts between the strains

The strain-specific response in the timing of the regenerative events in autografts might reflect inherent differences in the muscles themselves, as tissue culture studies clearly demonstrate faster myogenesis in SJL/J muscle (Maley et al. 1994, 1995) in the absence of exogenous host influences. Another possibility is that the muscles might have a different capacity to chemoattract or stimulate leucocytes due to factors produced by the damaged muscles themselves (Grounds & Davies, 1996). Alternatively, host influences might result in differences in the capacity of host leucocytes to respond to the injured muscle tissue: while it has been shown that this is not a function of the genotype of the bone marrow derived cells (Mitchell et al. 1995), it might reflect some modulation by the host environment of the leucocyte response. All these possibilities were tested in the allograft experiments.

The results of the cross transplantation (allograft) experiments clearly show that, in this situation, it is the host environment that determines the speed of the regenerative response. BALB/c grafts in SJL/J hosts had myotubes present at 3 d as was seen for the SJL/J autografts, and leucocyte infiltration was always faster in the SJL/J hosts regardless of the strain of the donor graft. The converse was seen in the BALB/c hosts, where leucocyte infiltration and myotube formation was slower (than for SJL/J hosts) for both SJL/J and BALB/c grafts. The simplest explanation for these results is that some aspect of the SJL/J host environment affects leucocytes so that they are more avaricious (either ‘pre-activated’ or more rapidly activated) than those in the BALB/c host environment. In our experiments, it appears to be the speed or efficiency of the leucocyte response in the host that controls the rate of new muscle formation.

This host influence is reminiscent of differences between skeletal muscle regeneration in old and young animals where it is well recognised that regeneration is slower in old hosts (McGeachie & Grounds, 1995) and cross transplantation experiments in rats show that the pattern of regeneration is dictated by the age of the host, rather than the muscle graft (Carlson & Faulkner, 1989). Changes in cytokine and hormonal states with age may also affect the leucocyte response in old hosts (Cannon, 1995).

The ‘accelerated leucocyte response’ in the SJL/J host mice may reflect the immune condition that underlies the ability to provoke autoimmune diseases, such as myositis and encephalitis, in this strain and the inherent capacity of older SJL/J mice to develop myositis (Rosenberg et al. 1988; Rosenberg, 1993).

At 5 d after transplantation, it was noted that more leucocytes were present in the allografts in both hosts than in any autografts at this time. It was considered that this represented an early immunological reaction by the hosts to the foreign muscle tissue. Furthermore, more myotubes were present in these allografts than in any autografts at 5 d. This accelerated myotube formation was attributed to the presence of the large numbers of leucocytes, particularly activated macrophages, which are known to produce growth factors that stimulate myogenesis.

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