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Effects of gliotoxin on Langerhans' cell function: contact hypersensitivity responses and skin graft survival

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SUMMARY

Dendritic Langerhans' cells (LC), which are essential for the induction of cutaneous immunity, express high concentrations of class II major histocompatibility (MHC) glycoproteins (Ia in the mouse) on their plasma membrane. Application of gliotoxin, a member of the epipolythiodoxopiperazine (ETP) group of fungal metabolites, reduces epidermal LC density and alters their morphology from highly dendritic to a more rounded form. Here we demonstrate that gliotoxin also alters LC function, reducing contact hypersensitivity (CHS) responses due to the development of suppressor cells, and enhancing C57BL tail skin graft survival on BALB/c recipients. The reduction in LC density following gliotoxin application was shown to enchance skin graft survival, by reducing the concentration of Ia antigens within the graft, by using congenic mouse strains: B10.A(2R) × B10.A, differing only at H-2D, and B10.A(2R) × B10.A(4R), differing only at H-2 I-E. Treatment of B10.A(2R) tail skin with gliotoxin for 1 week did not affect its survival when grafted onto H-2D-disparate B10.A mice, whereas, when grafted onto H-2 I-E-disparate B10.A(4R) hosts, the grafts were not only accepted permanently, but induced specific unresponsiveness. It is concluded that gliotoxin has a marked effect on LC function, inhibiting CHS responses by the induction of suppressor cells and prolonging graft survival between H-2-disparate and congenic mouse strains.

INTRODUCTION

Langerhans' cells (LC) are dendritic bone marrow-derived cells which form a network in the suprabasal epidermis (Halliday & Muller, 1984) and are responsible for the initiation of immune responses against cutaneous foreign antigens (Stingl, Tamaki & Katz, 1980). They are normally the only cells within the epidermis to express the class II major histocompatibility (MHC) glycoproteins (Rowden, 1981). These glycoproteins are essential for antigen presentation to T lymphocytes (Ununue *et al.*, 1984), and are only present on limited subpopulations of cells of the immune system, such as dendritic cells, macrophages, B cells and activated T cells (Streilein & Bergstresser, 1980).

Skin can be depleted of detectable LC by various agents, including short wavelength ultraviolet radiation (UVB; Odling, Halliday & Muller, 1987a), carcinogens such as 9, 10-dimethyl-1, 2-benzanthracene (DMBA; Halliday & Muller, 1986) and immunosuppressive agents such as glucocorticosteroids (Ashworth, Booker & Breathnatch, 1988; Halliday, Knight & Muller, 1986) and cyclosporin A (Halliday *et al.*, 1986). It is thought that UVB and carcinogens such as DMBA deplete LC from the epidermis, since 8–9 weeks is required for LC to repopulate the epidermis following cessation of treatment (Odling *et al.*, 1987a; Halliday *et al.*, 1987). In contrast, at least some corticosteroids probably modulate LC surface antigens, such as adenosine triphosphatase (ATPase; Lynch, Gurish & Daynes, 1981) and class II MHC glycoproteins (Nordlund, Ackles & Lerner, 1981), resulting in an apparent LC depletion, as LC return to normal numbers in the epidermis within 4–5 days. We have previously reported (McMinn *et al.*, 1990) that gliotoxin causes a depletion of LC from the epidermis that is associated with ultrastructural evidence of LC damage, and that LC require 13 weeks to repopulate the epidermis; suggesting that gliotoxin is retained within the epidermis for a prolonged period.

Gliotoxin is a member of the epipolythiodioxopiperazine (ETP) class of fungal metabolites and has been isolated from cultures of *Aspergillus fumigatus* (Mullbacher, Waring & Eichner, 1985). It is known to have a wide range of antibacterial, anti-viral and anti-fungal activity (Taylor, 1971). Recently, gliotoxin has been shown to have a marked antiphagocytic effect on macrophages (Mullbacher *et al.*, 1985) and other immunomodulating properties, including inhibition of proliferation of lymphocytes in mixed lymphocyte cultures (Eichner *et al.*, 1986). It also results in a failure to produce cytotoxic T cells when stimulator cells are treated with gliotoxin prior to the commencement of culture (Waring, Eichner & Mullbacher, 1988).

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Table 1. H-2 genotypes of inbred mouse strains

	MHC region			
Mouse strain	H-2K	I-A	I-E	H-2D
BALB/c	d	d	d	d
C57BL	b	b	b	b
B10.A	k	k	k	k
B10.A(4R)	k	k	b	b
B10.A(2R)	k	k	k	b

The function of LC in initiating contact hypersensitivity (CHS) responses is now well established (Shelley & Juhlin, 1976). LC bind cutaneous antigens and migrate to local lymph nodes where they present the antigen, in association with class II MHC glycoproteins, to T cells, inducing a CHS response (Halliday & Muller, 1984). Contact sensitizing agents such as dinitrofluorobenzene (DNFB) applied to skin depleted of LC by UVB (Noonan, De Fabo & Kripke, 1981) or DMBA (Halliday & Muller, 1986) results in antigen-specific tolerance, due to the induction of suppressor cells (Halliday & Muller, 1987; Halliday, Cavanagh & Muller, 1988a).

The class II MHC glycoproteins are strong transplantation antigens; skin grafts exchanged between H-2-recombinant congenic mouse strains, disparate only for the class II MHC antigens, are rejected (Klein, 1977; Mitchison, 1979). It has been shown that skin allograft survival is prolonged by removing the outer layer of donor skin, including LC, by repeated stripping with cellophane tape (Streilein, Lonsberry & Bergstresser, 1982). More recently it has been shown that treatment of donor skin with various doses of UVB (Odling *et al.*, 1987a) or DMBA (Odling, Halliday & Muller, 1987b) prolongs skin allograft survival. It has also been demonstrated that when grafts are exchanged between H-2-congenic mouse strains which differ only at the I-E region, donor skin treated with DMBA prior to grafting is permanently accepted by the recipients, whereas grafts exchanged between strains differing only at the H-2D region are promptly rejected, and DMBA alone has no significant effect on graft survival (Odling *et al.*, 1987b). These experiments suggest that class II MHC glycoproteins are major determinants of allogeneic skin graft rejection.

We have previously shown that gliotoxin depletes class II MHC-positive LC from murine epidermis (McMinn *et al.*, 1990). To define the functional effects of gliotoxin on LC, contact hypersensitivity and allogeneic skin graft survival were investigated.

MATERIALS AND METHODS

Animals

B10.A, B10.A(2R) and B10.A(4R) mice were obtained from the Animal Resources Centre, Perth, WA. C57BL and BALB/c mice were supplied by the Central Animal House, Unversity of Tasmania, Australia. The H-2 genotypes of these mice are shown in Table 1. All mice were used with the approval of the Animal Ethics Committee. In each experiment donors and recipients were sex matched; they were kept in groups of six in standard boxes with food and water *ad libitum*.

LC depletion by gliotoxin

Gliotoxin was derived from the culture supernatants of the fungus *Aspergillus fumigatus* (Mullbacher *et al.*, 1985) and was a gift of Dr P. Waring, John Curtin School of Medical Research, Australian National University, Canberra, Australia. Gliotoxin was dissolved in acetone, diluted to the appropriate concentrations and stored at $-20°$.

For contact sensitivity experiments, 20 μl of gliotoxin or solvent were applied to the shaved dorsal trunk or abdomen of BALB/c mice. For grafting studies, 100 μl of gliotoxin or solvent were applied to the dorsal surface of the proximal 2 cm of the tail skin of donor C57BL and B10.A(2R) mice. Three treatments were given 48 hr apart, and the tail skin was removed for grafting 48 hr following the final gliotoxin treatment.

LC identification

LC in B10.A(2R) mice were quantified by staining epidermal sheets for membrane-bound class II MHC glycoproteins, as described previously (Halliday *et al.*, 1988b; Halliday, Dickenson & Muller, 1989). In brief, ear skin supported by cellophane tape applied to the stratum corneum was incubated for 2 hr at 37° in isotonic phosphate-buffered saline (PBS) containing 20 mM ethylene diamine tetracetic acid (EDTA), pH 7·3, in order to facilitate the epidermis being separated from the dermis in a single sheet.

Ia-positive LC in the epidermal sheets were visualized by light microscopy and identified by their dendritic morphology and staining by indirect immunoperoxidase with TIB-92 cell supernatant containing anti-Ia^k monoclonal antibody (ATCC, Rockville, MD), followed by peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dakopatts, Glostrup Denmark) and 0·05% 3,3-diaminobenzidine (DAB; Sigma, St Louis, MO) in PBS (pH 7·3), containing one drop/10 ml hydrogen peroxide. The number of LC present in six microscopic fields was counted for each mouse, the field size being determined with a graticule. A total area of 4 mm^2 was counted per mouse, and the number of LC per mm^2 of epidermis calculated.

Contact sensitization

Mice were sensitized to 2,4-dinitrofluorobenzene (DNFB; Lot no. 44F-0533; Sigma) by applying 25 μl of 0·5% (v/v) DNFB in 4:1 acetone-olive oil to either shaved dorsal trunk or abdominal wall skin for two consecutive days (Toews, Bergstresser & Streilein, 1980).

Assessment of contact sensitivity

A contact sensitivity response was elicited by applying 20 μl of 0·2% DNFB in 4:1 acetone-olive oil to the dorsal surface of the right ear of mice 5 days following initial sensitization. The thicknesses of the challenged and unchallenged ears were measured 24 hr later with a spring-loaded engineers' micrometer. The contact sensitivity response was expressed as a percentage increase in ear thickness, as calculated by the formula:

$$\frac{\text{thickness of challenged ear} - \text{thickness ofunchallenged ear}}{\text{thickness of unchallenged ear}} \times 100.$$

Adoptive transfer of suppressor cells

Suppressor T cells were detected by their ability, upon transfer into naive syngeneic hosts, to inhibit the host response to sensitization (Greene *et al.*, 1979). Spleens removed from donors were gently teased into Hanks' balanced salt solution (HBSS; pH 7·3) and washed three times by centrifugation; 5×10^7 donor splenocytes were injected into the tail vein of hosts that had been anaesthetized with 36 mg/kg body weight of sodium pentobarbitone (Ceva Chemicals Pty Ltd, Sydney, NSW, Australia). Mice were then sensitized with DNFB within 2 hr of spleen cell transfer.

Skin grafting

Control and gliotoxin-treated donor C57BL or B10.A(2R) mice were killed by cervical dislocation, and tail skin was grafted onto the left thoracic wall of BALB/c, B10.A or B10.A(4R) mice, as described by Billingham & Medawar (1951). Tail skin was peeled away, after making an incision around the base and along the full length of the tail, placed dermal side down on saline-moistened filter paper, and 3×8 mm sections were prepared from the proximal region.

Recipient mice were anaesthetized with sodium pentobarbitone and their thoracic and abdominal body wall clipped free of hair. The mice were held in an extended position to ensure that the skin was taught, and the graft bed was prepared by surgical removal of skin to the level of the fascia that overlies the panniculus carnosus. Donor skin was placed flat onto the graft bed, leaving a 1·5 mm margin around the graft, and bandaged into position by successive layers of tulle gras (Jelonet, Smith and Nephew, Victoria, Australia), elastoplast (Smith and Nephew) and plaster of Paris bandage (Gypsona, Smith and Nephew). Bandages were removed 6 days later, and the grafts were examined daily using a hand lens.

Grafts were initially recorded as accepted if they were vascularized and healthy; rejection was recorded as the day of total skin graft loss. Prior to rejection, grafts lost their pink colour and became grey, often flaking away from the graft bed.

Statistics

An unpaired Student's *t*-test was used to compare the responses between different groups for both contact sensitivity testing and skin grafting.

RESULTS

Contact sensitivity responses

The contact hypersensitivity response of BALB/c mice following application of DNFB to solvent- or gliotoxin-treated skin was found to be highly sensitive to inhibition by low concentrations of gliotoxin (Table 2). All concentrations of gliotoxin assessed reduced the CHS response to DNFB, from a control value of 56·5% increase in ear thickness to values between 35·1% and 23·8%, in a dose-dependent manner. In these experiments, controls consisted of a group of mice treated with acetone prior to sensitization with 0·5% DNFB. The acetone treatment did not reduce the CHS response, as mice sensitized via untreated skin developed a 46·5% increase in ear thickness. A negative control of an unsensitized group of mice challenged on the right ear only without sensitization did not respond, indicating that CHS, and not non-specific irritation, was being assessed (Table 2).

Table 2. Contact sensitivity response of BALB/c mice sensitized to DNFB through gliotoxin-treated skin

Treatment group	Mean % increase in ear thickness (SD)*	Significance of difference†
Sensitized and challenged only‡	46·5 (28·0)	—
Ear challenge only§	2·8 (6·9)	—
Solvent	56·5 (9·8)	—
Gliotoxin 10^2 ng/ml	35·1 (7·1)	$P<0.01$
Gliotoxin 10^3 ng/ml	27·0 (7·8)	$P<0.001$
Gliotoxin 10^4 ng/ml	23·8 (9·9)	$P<0.001$

* Six mice per group.
† Unpaired Student's *t*-test; compared with solvent-treated group.
‡ Mice sensitized and challenged to DNFB only.
§ Mice challenged with DNFB on the right ear only.

In order to determine whether gliotoxin inhibited contact sensitivity by systemic suppression, a group of mice was treated with gliotoxin on the dorsal surface prior to sensitization with DNFB on the ventral abdominal wall. Upon challenge, these mice gave a 48·6% increase in ear thickness, which was not significantly different from the acetone-treated control group (34·3%).

Suppressor T-lymphocyte activation

To define the nature of the inhibition of the CHS response to DNFB following sensitization through gliotoxin-treated skin, spleen cells from mice that had been treated with gliotoxin prior to DNFB sensitization and challenge were injected into the tail veins of naive syngeneic host mice 9 days following the initial sensitization of donor mice. The recipient mice were sensitized to DNFB within 2 hr of cell transfer and challenged, as described previously.

Spleen cells from mice that had been treated with various doses of gliotoxin prior to DNFB sensitization through treated skin, significantly decreased the ability of host mice to respond to sensitization upon transfer into naive host mice. These groups gave significantly lower CHS responses than hosts of spleen cells from untreated mice (Table 3). Spleen cells from mice treated with solvent and DNFB did not reduce the CHS response when transferred into host mice, as this response was not significantly different from sensitization following transfer of spleen cells from untreated donors.

LC enumeration

To quantify the effect of gliotoxin on B10.A(2R) mouse LC, the epidermis was stained for class II MHC glycoproteins by indirect immunoperoxidase, and LC identified by light microscopy. Untreated dorsal B10.A(2R) ear contained an average of $652·9 \pm 40·3$ epidermal LC/mm^2 for six mice tested. Treatment with gliotoxin (10^4 ng/ml $\times$ 3, 48 hourly), followed by epidermal sheet preparation 48 hr following the final gliotoxin treatment, resulted in a significantly lower epidermal LC density of $467·3 \pm 28·2$ LC/mm^2 for six mice ($P<0.001$).

Table 3. Contact sensitivity response of host BALB/c mice sensitized to DNFB following adoptive transfer of spleen cells from donor syngeneic mice sensitized to DNFB through gliotoxin treated skin

Skin treatment of spleen cell donors	Mean % increase in ear thickness of recipients (SD)*	Significance of difference†
Untreated	35·5 (20·5)	—
Solvent	44·2 (13·8)	NS
Gliotoxin 10^2 ng/ml	17·7 (12·5)	$P<0·01$
Gliotoxin 10^3 ng/ml	14·0 (20·4)	$P<0·01$
Gliotoxin 10^4 ng/ml	16·6 (7·9)	$P<0·01$

* Six mice in each group.

† Unpaired Student's *t*-test; compared to untreated spleen cell donor group.

NS, not significant.

Table 4. Survival of C57BL mouse tail skin treated with gliotoxin and grafted onto BALB/c mice

Treatment of donors	Graft survival (days ± SD)	Significance of difference from Solvent-treated group*	No of mice in group
Solvent	8·8 (1·3)	—	10
Gliotoxin (10^3 ng/ml)	9·8 (1·7)	NS	11
Gliotoxin (10^4 ng/ml)	11·5 (2·5)	$P<0·001$	11
Gliotoxin (10^5 ng/ml)	10·7 (2·1)	$P<0·001$	12

* Unpaired Student's *t*-test.

NS, not significant.

Graft survival between histoincompatible mouse strains

Control C57BL tail skin grafted onto BALB/c mice, which differ across the entire H-2 locus (Table 1), had an average survival time of 8·8 days. After treatment with gliotoxin (10^4 or 10^5 ng/ml × 3, 48 hourly), C57BL tail skin grafted onto BALB/c recipients 48 hr following the final gliotoxin treatment had a significantly increased survival time. A gliotoxin concentration of 10^3 ng/ml did not result in improved allogeneic graft survival (Table 4).

Following initial gliotoxin (10^4 ng/ml) treatment, graft survival remained significantly prolonged ($P<0·01$) when grafting was delayed for a period of 5 weeks (10·5 ± 1·4 days) compared to solvent-treated controls (8·0 ± 1·3 days). When grafting was delayed for 10 weeks following gliotoxin treatment, graft survival (8·1 ± 0·7 days) did not differ significantly from solvent-treated control grafts (8·0 ± 1·3 days).

Graft survival between congenic mouse strains

In order to define this enhanced graft survival further, H-2-disparate congenic mouse strains were used. Gliotoxin treatment of B10.A(2R) tail skin did not enhance survival when grafted onto H-2D-disparate B10.A hosts (Table 5). In contrast, tail skin from gliotoxin-treated (10^4 ng/ml) B10.A(2R) mice grafted onto H-2 I-E-disparate B10.A(4R) hosts was not rejected over a 160-day period, despite similar solvent-treated grafts being rejected after 12·8 days (Table 5). After 160 days, a second solvent-treated, sex- and age-matched B10.A(2R) tail skin graft was placed onto the opposite thoracic body wall of these B10.A(4R) mice which had permanently accepted the gliotoxin-treated B10.A(2R) tail skin. Neither the original gliotoxin nor the solvent-treated grafts were rejected in five out of eight recipients over a 5-week period; three solvent-treated grafts were lost within 12 days; however, the original gliotoxin-treated grafts were unaffected.

In order to assess the specificity of the failure to reject the transplants, the five hosts that did not reject the gliotoxin- or solvent-treated grafts, received a third, solvent-treated, sex-matched BALB/c tail skin graft. These final BALB/c grafts, whose genotype differs from both B10.A(2R) and B10.A(4R) mice across the entire H-2 locus (Table 3), were rejected at an average of 9·2 ± 1·3 days. The gliotoxin- and solvent-treated B10.A(2R) grafts were unaffected by the rejection of the BALB/c grafts.

DISCUSSION

We have previously shown that gliotoxin depletes class II MHC-positive LC from BALB/c epidermis (McMinn *et al.*, 1990), associated with ultrastructural evidence of LC damage at low gliotoxin concentrations. Following cessation of gliotoxin treatment, LC remain depleted for 12–13 weeks, probably due to gliotoxin remaining within the epidermis for a prolonged time. In the present study we have shown that gliotoxin also depletes LC from B10.A(2R) epidermis to a level similar to BALB/c mice.

Depletion of epidermal LC by the epicutaneous application of gliotoxin resulted in a marked reduction in LC antigen-presenting cell function. Inhibition of the CHS response occurred at the lowest concentration of gliotoxin used (100 ng/ml), and the percentage inhibition of CHS was similar for all concentrations assessed—up to 10^4 ng/ml. Inhibition of the CHS response occurred only when sensitization to DNFB occurred through gliotoxin-treated skin. As sensitization on the ventral abdominal skin following gliotoxin application on the dorsal surface did not inhibit the CHS response, it is concluded that gliotoxin acted by a local rather than systemic effect on LC. This is in accordance with our previous observations relating to LC morphology (McMinn *et al.*, 1990).

Similar to previous studies using carcinogens (Halliday & Muller, 1987; Halliday *et al.*, 1988), we found that the inhibition of CHS by gliotoxin was due to the activation of transferrable splenic suppressor cells. Spleen cells from mice sensitized to DNFB through gliotoxin-treated skin, when transferred into naive syngeneic host mice, prevented sensitization of the host mice to DNFB; whereas spleen cells from mice sensitized to DNFB through solvent-treated skin had no effect on the development of contact sensitization in naive syngeneic host mice. Other studies have demonstrated that suppressor cells activated via LC-depleted skin are both antigen-specific and long-lived (Halliday & Muller, 1987), and are activated in the local lymph node by an epidermal antigen-presenting cell

Table 5. Survival of B10.A(2R) tail skin treated with gliotoxin and grafted onto B10.A and B10.A (4R) recipients

Treatment of donors	Recipient strain (MHC disparity)	Graft survival (days ± SD)	Significance of difference*	No. mice in group
Solvent	B10.A (H-2D)	18·7 (2·8)	—	7
Gliotoxin (10^4 ng/ml)	B10.A (H-2D)	19·6 (2·0)	NS	9
Solvent	B10.A (4R) (I-E)	12·8 (4·9)	—	10
Gliotoxin (10^4 ng/ml)	B10.A (4R) (I-E)	> 160	$P < 0.001$	8

* Unpaired Student's *t*-test; comparison between solvent and gliotoxin-treated groups

NS, not significant.

population (Halliday *et al.*, 1988) which presents antigen in an I-J-restricted manner (Halliday, Wood & Muller, 1990).

High concentrations of gliotoxin (10^4–10^5 ng/ml) applied to donor C57BL tail skin resulted in prolonged graft survival on BALB/c recipients, which differ across the entire H-2 locus. There was no significant improvement in graft survival when lower concentrations of gliotoxin were used, despite concentrations between 100 and 1000 ng/ml causing significant but small reductions in LC density (McMinn *et al.*, 1990) and producing marked reductions in CHS responses. This finding suggests that a critical reduction in LC density, and therefore class II MHC antigen density, is required for prolongation of allogeneic graft survival, which accords with other studies in which UVB (Odling *et al.*, 1987a) and the chemical carcinogen DMBA (Odling *et al.*, 1987b) have been used to deplete LC from the epidermis.

Grafting studies using H-2 recombinant congenic mouse strains have confirmed these findings. Gliotoxin-treated B10.A(2R) tail skin grafted onto B10.A recipients survive for a similar length of time as solvent-treated grafts. Since these mice are identical at the I region and differ only at the H-2D region, depletion of LC had no effect on graft survival. A previous study has shown that depletion of Ia antigens with anti-Ia antibodies has no effect on H-2D-incompatible congenic recombinant recipients (McKenzie, Hennings & Morgan, 1980). However, when gliotoxin-treated B10.A(2R) tail skin was transplanted onto B10.A(4R) recipients, which differ at the I-E locus only, the grafts were not rejected after 160 days, suggesting that they had been permanently accepted.

In this system, as LC were expressing the only transplantation antigen (I-E), depletion of LC from the graft reduced the Ia antigens within it, significantly reducing the only known antigenic disparity between the two strains. Furthermore, a second solvent-treated B10.A(2R) graft was accepted, indicating that the first gliotoxin-treated graft had induced tolerance to the I-E^k transplantation antigens within the graft. These findings are consistent with those of Odling *et al.* (1987b), using the chemical carcinogen DMBA. They argued that the residual LC in the DMBA-treated graft, and the subsequently reduced level of Ia antigenicity, may have delivered a low-dose tolerogenic signal to the host, resulting in the second solvent-treated B10.A(2R) graft being accepted as well. The unresponsiveness thus generated is specific, since the ability to reject a third, genetically unrelated (BALB/c) graft remains unaltered. The studies using congenic mouse strains suggest that LC act as a source of class II MHC transplantation antigens during graft rejection rather than functioning as antigen-presenting cells, since in this case gliotoxin would have also enhanced class I-disparate graft survival by reducing both the number and activity of LC from the graft.

However, the experiments in which grafting was delayed for increasing time intervals from gliotoxin application tend to contradict these findings. When grafting of C57BL tail skin was delayed for 5 weeks from the time of gliotoxin application, graft survival on BALB/c recipients was significantly improved. By the time 10 weeks had elapsed between gliotoxin application and grafting, graft survival had returned to control values. Following gliotoxin application, it has been shown that LC remain significantly depleted for 12–13 weeks, hence graft survival had decreased despite continuing low levels of LC within the graft, suggesting that the remaining LC had returned to normal function.

In conclusion, these results suggest that a combination of sufficient density of class II MHC antigens and adequate antigen-presenting cell function is required for efficient rejection of skin allografts. This is consistent with the two-signal model for T-cell activation (Lafferty, Prowse & Simeonovic, 1983), in which both recognition of allogeneic transplantation antigens and a second or subsequent 'signal' (such as a cytokine) from a functionally intact 'stimulator cell' within the graft are required for adequate T-cell activation and initiation of graft rejection. Dendritic cells, such as LC, within graft tissue have been proposed as stimulator cells, capable of providing both the antigenic and functional signals necessary for graft rejection (Batchelor, Welsh & Burps, 1978). Our findings are in agreement with this hypothesis.

The investigation of agents such as gliotoxin, which can preferentially inhibit the function of immunocompetent cells within the epidermis (or other organs), has potential clinical significance as this may allow inhibition of graft rejection without adversely affecting organ function, and hence could be used for pretreatment of donor tissue prior to grafting. Gliotoxin has been shown to greatly improve survival of allogeneic thyroid tissue grafts (A. Mullbacher, personal communication), foetal pancreatic islet cell grafts (Lissing, Tuch & Suranyi, 1988) and bone marrow transplantation (Mullbacher *et al.*, 1988), in which donor tissue has been incubated with gliotoxin prior to grafting. In the latter study, allogeneic marrow was grafted to

lethally irradiated recipients, all of which became fully reconstituted without the development of graft-versus-host disease. The ability to use an agent such as gliotoxin, which selectively inhibits immunogenic cells within a graft, is of great potential significance for the future of transplantation biology.

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