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Transfer of specific immunosuppression of graft rejection using lymph from tolerant liver-grafted rats

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Summary. The effect on graft rejection of lymph from rats rendered tolerant of donor antigens by liver transplantation has been studied. Transfer, by daily intravenous injection, of lymph from PVG rats grafted with DA livers prolonged the survival of DA skin, kidney and heart grafts in normal PVG recipients. The effect was specific for the antigens of the liver donor. Suppression was short-term only; thus, after lymph injections were stopped, rejection occurred with a time course approximating a normal first-set reaction. The result suggests a reversible interference by materials in the tolerant lymph with early stages of sensitization of the recipients.

INTRODUCTION

A remarkable feature of liver transplantation is that, in certain species, allografts are frequently accepted without the usual need for immunosuppressive treatment of the recipients. This phenomenon was first discovered in the pig (Cordier *et al.*, 1965; Peacock & Terblanche, 1967; Calne *et al.*, 1967) and has more recently been demonstrated in certain donor-recipient combinations of inbred strains of rat (Kamada, Brons & Davies, 1980; Houssin *et al.*, 1980). For example, DA livers grafted orthotopically into PVG rats are never rejected, despite the barrier of the major histocompatibility complex (MHC), while grafts of other organs (skin, heart, kidney) between these strains are rejected in the expected acute manner (Kamada *et al.*, 1980). It is also clear that an allogeneic liver induces a state of specific, systemic immunological tolerance to donor MHC antigens; this is demonstrated by the permanent acceptance by liver-grafted pigs and rats of skin, heart and kidney grafts of the same strain as the liver donor, while tissues of third-party strains are rejected promptly (Calne *et al.*, 1969; Kamada, Davies & Roser, 1981a; Kamada & Wight, 1984; Kamada, 1985). Moreover, a liver graft is even able to overcome the effects of priming by donor antigens and to convert a state of immunological memory into one of specific transplantation tolerance (Kamada, Davies & Roser, 1981b).

In previous publications, we have described adoptive transfer experiments which show that liver-induced tolerance is accompanied by the specific deletion of alloreactive lymphocyte clones from the recipient's immunological repertoire, with no evidence for T-cell mediated suppression (Davies, Kamada & Roser, 1983; Roser *et al.*, 1983). In this communication, we report that donor-specific immunosuppression can be transferred to normal PVG rats by lymph from PVG animals grafted with DA livers, but that, unlike liver transplantation itself, the suppression transferred in this way does not lead to a state of permanent tolerance.

Abbreviations: GVH, graft versus host reaction; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; MST, mean survival time; TD, thoracic duct.

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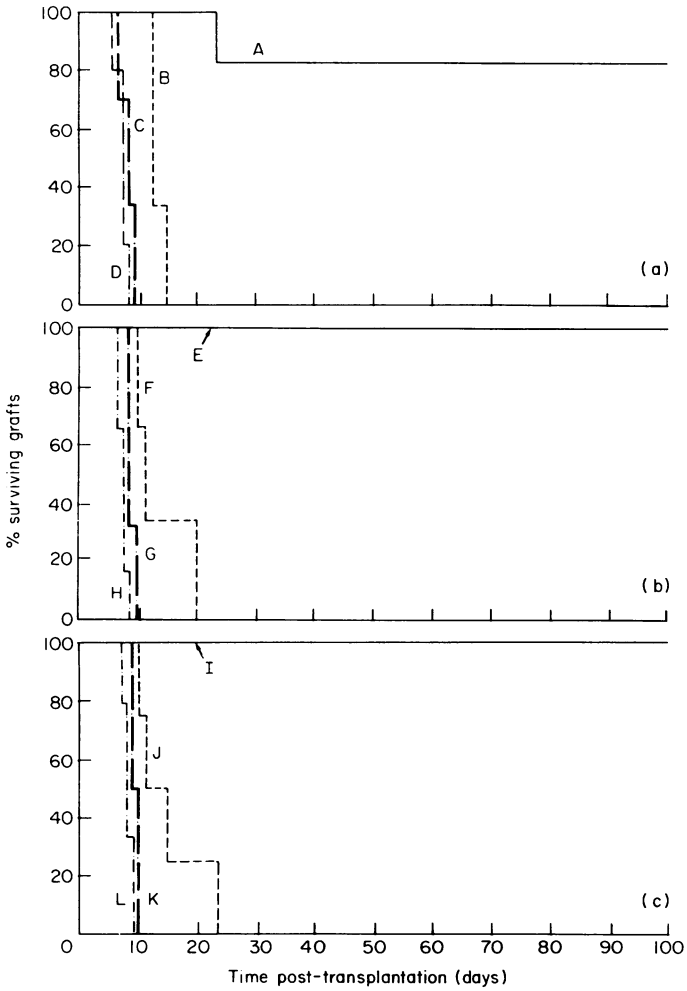

Figure 1. PVG rat recipients of orthotopic DA liver grafts accept grafts of DA skin, heart or kidney (n = number in group; result as mean survival time ± standard deviation). (a) Skin. Group A: survival of DA skin grafts on PVG rats grafted orthotopically 15 days earlier with DA liver ($n=6$; 87·1 ± 28·7 days). Group B: survival of LEW skin grafts on PVG rats grafted 15 days earlier with DA liver ($n=3$; 13·7 ± 0·9 days). Group C: survival of DA skin grafts on normal PVG rats ($n=8$; 8·1 ± 0·7 days). Group D: survival of LEW skin grafts on normal PVG rats ($n=5$; 8·0 ± 0·6 days). (b) Heart. Group E: survival of heterotopic DA heart grafts on PVG rats grafted orthotopically at the same time with DA liver ($n=6$; > 100 days). Group F: survival of WAG heart grafts on PVG rats grafted at the same time with DA liver ($n=6$; 15·0 ± 6·8 days). Group G: survival of DA heart grafts on normal PVG rats ($n=6$; 8·3 ± 0·5 days). Group H: survival of WAG heart grafts on normal PVG rats ($n=6$; 7·8 ± 0·7 days). (c) Kidney. Group I: survival of DA renal transplants in PVG rats grafted orthotopically at the same time with DA liver ($n=6$; > 100 days). Group J: survival of WAG renal transplants in PVG rats grafted at the same time with DA liver ($n=4$; 15·0 ± 5·5 days). In this group, histological examination at autopsy revealed signs of severe rejection of the WAG kidney graft, but only mild rejection in the DA liver graft. Group K: survival of DA renal transplants in normal PVG rats ($n=6$; 9·5 ± 0·5 days). Group L: survival of WAG renal transplants in normal PVG rats ($n=6$; 9·2 ± 0·7 days).

MATERIALS AND METHODS

Animals

PVG ($RT1^c$), DA ($RT1^a$), LEW ($RT1^l$), AO ($RT1^u$) and WAG ($RT1^u$) rats were obtained from OLAC (1976) Ltd, Bicester, Oxfordshire.

Transplantation

(a) Liver. Orthotopic liver transplantation (DA donors, PVG recipients) was performed by our published technique (Kamada & Calne, 1983). Rejection does not occur in this combination (Kamada *et al.*, 1980).

(b) Skin. Full thickness orthotopic skin grafting was carried out according to the method of Roser & Ford (1972). Grafts were inspected daily after the seventh day and scored as rejected on the first day of complete epithelial necrosis.

(c) Heart. Heterotopic auxiliary heart grafting was performed in the neck using the method of Heron (1971). Rejection was defined by cessation of heart beat on neck palpation and confirmed by histological examination of the grafts.

(d) Kidney. Right or left orthotopic renal transplantation was performed using our previously published technique (Kamada, 1985). Technical success of vascular and ureteric anastomoses was checked 5 days after grafting, and nephrectomy of the host's own remaining kidney carried out at the same time. Rejection was defined by the day of death of the recipient and confirmed by histology.

Lymph

Thoracic duct lymph was collected from liver-grafted and normal rats by cannulation of the thoracic duct (Bollman, Cain & Grindlay, 1948), 20–70 ml being obtained from each rat overnight, with only a slight dilution by 5 ml Dulbecco's phosphate-buffered (A and B) saline in the collecting flask. After removing cells by centrifugation, a single pool of the lymph from 20 liver-grafted rats was prepared for use in all experiments and stored at −20°.

Lymph inoculations of 2–3 ml daily were given to PVG recipients via the tail vein, starting on the day of grafting with skin, kidney or heart; the amounts and duration of treatment are specified in the results.

RESULTS

PVG recipients of DA liver grafts are specifically tolerant of DA antigens

Apart from controls, all the rats used as donors of lymph in these experiments were PVG recipients of DA liver grafts. Such animals have a greater than 95% long-term survival, over 1000 allogeneic liver grafts having been performed in this combination in our laboratory (Kamada & Calne, 1983). Figure 1 shows that a state of specific systemic tolerance was induced

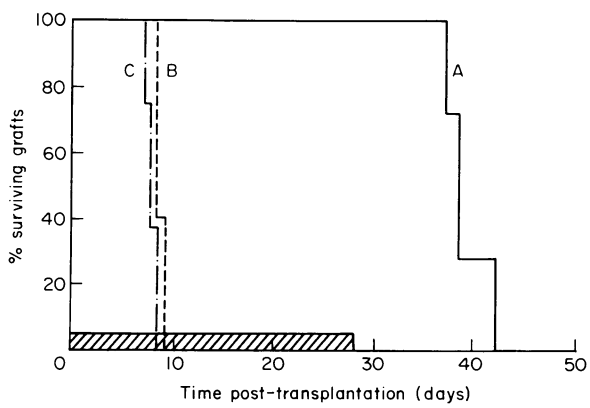

Figure 2. Effect on skin graft survival of lymph from PVG rats grafted with DA livers. Group A: survival of DA skin grafts on PVG rats injected daily i.v. for 28 days with 3 ml of TD lymph from PVG rats carrying orthotopic DA liver grafts ($n=7$; $38{\cdot}9 \pm 2{\cdot}0$ days). Group B: survival of AO skin grafts on PVG rats treated as in Group A ($n=5$; $8{\cdot}4 \pm 0{\cdot}5$ days). Group C: survival of DA skin grafts on untreated PVG recipients ($n=8$; $8{\cdot}4 \pm 0{\cdot}5$ days). Cross-hatched area indicates period of lymph administration.

against DA antigens in the PVG recipients of DA livers, such that subsequent grafts of DA skin (Group A), or simultaneous DA heart grafts (Group E) or kidney grafts (Group I), survived indefinitely. [Elsewhere, we have shown that an interval of 5–15 days is required to fully establish tolerance against skin grafts (Kamada *et al.*, 1981a).] Grafts of these organs from third-party donors were rejected with only a small (but significant) prolongation of survival (Groups B, F and J, respectively). Animals used as lymph donors received their livers in parallel with the groups shown in Fig. 1.

Specific prolongation of DA skin graft survival with TD lymph from liver-grafted PVG rats

Thoracic duct lymph was taken from PVG rats 30–60 days after grafting of DA liver, a time when tolerance of DA was fully established, and injected into normal PVG rats bearing DA skin grafts. Three ml of lymph was administered daily for 28 days, starting on the day of skin grafting. Figure 2 shows that DA skin grafts (Group A), but not third party (AO) grafts (Group B), showed a considerably prolonged survival as a result of lymph transfer compared with untreated controls (Group C); mean survival times were $38{\cdot}9 \pm 2{\cdot}0$ days (Group A), $8{\cdot}4 \pm 0{\cdot}5$ days (Group B) and $8{\cdot}2 \pm 0{\cdot}4$ days (Group C). It is evident that the DA grafts remained intact so long as the lymph was administered, and that acute rejection commenced a few days after lymph transfer was suspended, with a time course approximating that expected of a first-set reaction. There were some signs of destructive attack on the graft (loss of hair and atrophy) during the period of lymph administration, but the epithelium remained intact. Controls of lymph from isografted rats (PVG liver into PVG) had no effect on survival of skin grafts (result not shown).

Specific prolongation of DA heart graft survival with TD lymph from liver-grafted PVG rats

Fully allogeneic heterotopic (auxiliary) heart grafting was performed using the DA to PVG or AO (third party) to PVG combinations. PVG rats with DA or AO heart grafts were injected daily for 7 days i.v. with 2 ml of lymph from PVG recipients of DA livers; lymph transfer began on the day of heart grafting. The survival of these grafts is shown in Fig. 3. DA heart grafts showed specifically prolonged survival with lymph transfer (MST $13{\cdot}8 \pm 1{\cdot}8$ days, Group A) compared with the third-party control ($8{\cdot}2 \pm 0{\cdot}4$ days, Group C) or untreated recipients of DA heart grafts ($8{\cdot}3 \pm 0{\cdot}5$ days, Group D); moreover, lymph from DA isografted rats (DA liver into DA) was without effect (Group B). Thus, as with skin grafts (above), DA hearts were rejected soon after lymph treatment was terminated and at about the expected first-set rate.

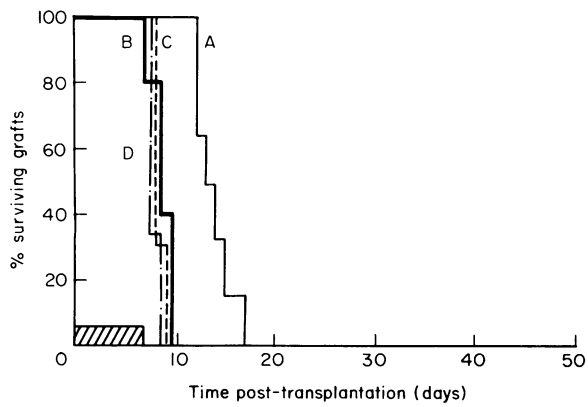

Figure 3. Effect on allogeneic heart graft survival of lymph from PVG rats grafted with DA livers. Group A: survival of heterotopic DA heart grafts on PVG rats injected daily i.v. for 7 days with 2 ml TD lymph from PVG rats carrying orthotopic DA liver grafts ($n=6$; $13{\cdot}8 \pm 1{\cdot}8$ days). Group B: survival of DA heart grafts on PVG rats treated similarly to Group A, but with TD lymph from DA rats grafted with DA livers ($n=5$; $8{\cdot}2 \pm 0{\cdot}7$ days). Group C: survival of AO heart grafts on PVG rats treated as in Group A ($n=5$; $8{\cdot}2 \pm 0{\cdot}6$ days). Group D: survival of DA heart grafts on untreated PVG recipients ($n=6$; $8{\cdot}3 \pm 0{\cdot}5$ days). Cross-hatched area indicates period of lymph administration.

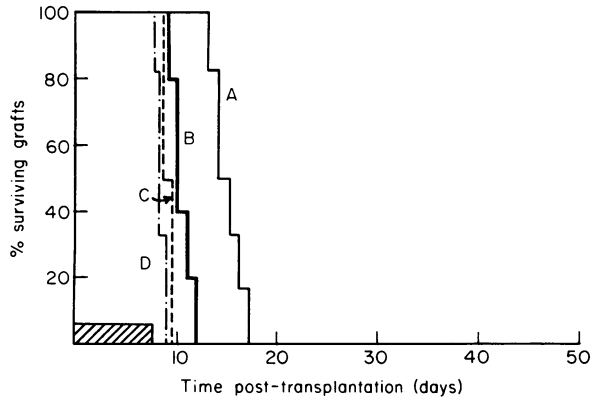

Figure 4. Effect on survival of renal allografts of lymph from PVG rats grafted with DA livers. Group A: survival of DA kidney grafts in PVG rats injected daily i.v. with 2 ml TD lymph from PVG rats carrying orthotopic DA liver grafts ($n=6$; $14{\cdot}8 \pm 1{\cdot}3$ days). Group B: survival of WAG kidney grafts in PVG rats treated as in Group A ($n=5$; $10{\cdot}4 \pm 1{\cdot}0$ days). Group C: survival of DA kidney grafts in untreated PVG recipients ($n=6$; $9{\cdot}5 \pm 0{\cdot}5$ days). Group D: survival of WAG kidney grafts in untreated PVG recipients ($n=6$; $9{\cdot}2 \pm 0{\cdot}7$ days). Cross-hatched area indicates period of lymph administration.

Specific prolongation of DA kidney graft survival with TD lymph from liver-grafted PVG rats

Orthotopic renal transplantation was performed in the combination of DA kidney into PVG recipient, with WAG into PVG as the third-party specificity control (Fig. 4). TD lymph from PVG rats grafted with DA livers was administered i.v. for 7 days (2 ml per day). Once again, survival was prolonged in donor-specific fashion, i.e. in PVG recipients of DA kidneys (MST $14{\cdot}8 \pm 1{\cdot}3$ days, Group A), compared with either third-party, lymph-treated controls ($10{\cdot}4 \pm 1{\cdot}0$ days, Group B) or untreated recipients ($9{\cdot}2 \pm 0{\cdot}7$ days, Group C). Rejection again commenced a few days after lymph treatment ended.

DISCUSSION

In the rat combination of DA to PVG (and vice versa), allogeneic liver has a remarkable capacity for tolerance induction and immunosuppression. Not only do liver grafts survive indefinitely, but a state of specific systemic unresponsiveness to donor MHC antigens is induced which allows unhindered acceptance of other organs from the same donor (Fig. 1). Elsewhere, we have also shown that liver transplantation between these strains can reverse the sensitization produced by skin grafting, converting it to unresponsiveness (Kamada *et al.*, 1981b), and can rapidly abrogate ongoing heart rejection at an advanced stage in the process (Kamada & Wight, 1984). The immunological events in the PVG recipient of a DA liver are doubtless complex. Initially, histological signs of rejection can be seen in the grafted liver, but these eventually disappear, and after 3–4 months the organ has almost returned to normal (Kamada *et al.*, 1983). The tolerant state is accompanied by specific deletion of DA-reactive T cells required for graft rejection, but not those involved in MLR or GVH reactions (Davies *et al.*, 1983; Kamada, 1982); moreover, adoptive transfer experiments provide no evidence for T-cell mediated suppression (Davies *et al.*, 1983). Antibodies both against class I (A region) and class II (B region) antigens of the rat RT1 complex can be demonstrated in the serum of PVG recipients at various times after grafting (N. Kamada, personal observation). Free class I antigen is also present in the serum, as demonstrated by blocking of the binding of monoclonal anti-RT1A antibodies to DA red cells (Kamada *et al.*, 1981a). Thus, while clonal deletion is the most obvious explanation for tolerance in these animals, other mechanisms of immunosuppression or enhancement may also operate.

In the experiments described here, it has been shown that lymph from liver-allografted PVG rats transfers suppression of graft rejection to normal PVG recipients. The lymph was given repeatedly from the day of grafting, and equivalent effects were observed on fully allogeneic grafts of skin, heart and kidney. This

suppression has two important characteristics: (i) it is specific for the antigens of the liver donor (DA), grafts from third-party donors being totally unaffected, and (ii) it is short-term and reversible, lasting only for the period of administration of the lymph. After lymph transfer was terminated, the rejection process followed quite closely the time course expected of a normal first-set reaction, suggesting that suppression may have been due to prevention of sensitization of the recipient (afferent inhibition).

It is clear that lymph from liver-allografted rats does not transfer the state of tolerance which follows liver transplantation itself. Rather, its effects invite comparison with the phenomenon of enhancement, in which graft survival is prolonged by administration of anti-donor alloantibodies around the time of grafting, or preimmunization with donor alloantigens (or both) (Stuart, Weiss & Fitch, 1979). Enhancement is obtained particularly readily in the rat, where rejection of renal allografts has often been prevented entirely by a single injection of antibody (Stuart *et al.*, 1979). Renal and heart allografts are more easily enhanced in this way than skin, which is generally a difficult organ to protect. In the present experiments with lymph, however, long-term survival of kidney, heart or skin was not observed, even though inocula were given over several days. Nor was there any difference in effect on these three organs: all survived while lymph was administered and were rejected a few days after administration was stopped.

A number of mechanisms can be envisaged whereby temporary specific suppression of rejection might be achieved by lymph from liver-grafted donors. Antibody, antigen and complexes are obvious candidates as immunosuppressive agents; antibody (anti-MHC or anti-idiotypic) would seem to be the most likely in lymph, though the presence of free or complexed DA antigens cannot be discounted. However, as noted above, the effects of lymph differ markedly from the enhancement which antibody usually produces in the rat. Free antigen is unlikely to be the sole agent, since repeated attempts to prolong survival of DA grafts with normal DA serum, shown to contain free class I alloantigen (Kamada *et al.*, 1981a), were uniformly unsuccessful (N. Kamada, personal observation), as was the use of lymph from DA isografted rats (Fig. 3). The presence of antigen-specific factors from suppressor T cells is another possibility, though adoptive transfer of thoracic duct lymphocytes from liver grafted rats into irradiated, skin-grafted recipients has failed to reveal any evidence for suppressor T cells (Davies *et al.*, 1983; Roser *et al.*, 1983). Thus, at present, the nature of the immunosuppressive agent is unresolved and is currently the subject of further investigation.

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REFERENCES

BOLLMAN J.L., CAIN J.C. & GRINDLAY J.H. (1948) Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. *J. Lab. clin. Med.* **33,** 1349.

CALNE R.Y., DAVIS D.R., HADJIYANNAKS E., SELLS R.Y., WHITE D., HERBERTSON B.M., MILLARD P.R., JOYSEY V.C., DAVIES D.A.L., BINNS R.M. & FESTENSTEIN H. (1969) Induction of immunological tolerance by porcine liver allografts. *Nature (Lond.)*, **223,** 472.

CALNE R.Y., WHITE H.J.O., YOFFA D.E., BINNS R.M., MAGIN R.R., HERBERTSON B.M., MILLARD P.R., MOLINA V.P. & DAVIS D.R. (1967) Prolonged survival of liver transplants in the pig. *Br. Med. J.* **2,** 478.

CORDIER G., GARNIER H., CLOT J.P., CLAMEZ P., CORIN J.P., NIZZA M. & LEVY R. (1965) La greffe de foie orthotopique chez le porc; premiers resultats. *Mem. Acad. Chir.* **92,** 27.

DAVIES H.FF.S., KAMADA N. & ROSER B.J. (1983) Mechanisms of donor-specific unresponsiveness induced by liver grafting. *Transplant. Proc.* **15,** 831.

HERON I. (1971) A technique for accessory cervical heart transplantation in rabbits and rats. *Acta Path. Microbiol. Scand.* **79,** 366.

HOUSSIN D., GIGON M., FRANCE D. & BISMUTH H. (1980) Specific transplantation tolerance induced by spontaneously tolerated liver allograft in inbred strains of rats. *Transplantation*, **29,** 418.

KAMADA N. (1982) Tolerance induced by liver transplantation in the rat. *Ph.D. Thesis.* University of Cambridge, U.K.

KAMADA N. (1985) A description of cuff techniques for renal transplantation in the rat—use in studies of tolerance induction during combined liver grafting. *Transplantation*, **39,** 93.

KAMADA N., BRONS G. & DAVIES H.S. (1980) Fully allogeneic liver grafting in rats induces a state of systemic nonreactivity to donor transplantation antigens. *Transplantation*, **29,** 429.

KAMADA N. & CALNE R.Y. (1983) A surgical experience with 530 liver transplants in the rat. *Surgery*, **93,** 64.

KAMADA N., DAVIES H.S. & ROSER B. (1981a) Fully allogeneic liver grafting and the induction of donor-specific unreactivity. *Transpl. Proc.* **13,** 837.

KAMADA N., DAVIES H.S. & ROSER B. (1981b) Reversal of transplantation immunity by liver grafting. *Nature (Lond.),* **292,** 840.

KAMADA N., DAVIES H.S., WIGHT D.G.D., CULANK L. & ROSER B. (1983) Liver transplantation in the rat: biochemical and histological evidence of complete tolerance induction in non-rejector strains. *Transplantation,* **35,** 304.

KAMADA N. & WIGHT D.G.D. (1984) Antigen-specific immunosuppression induced by liver transplantation in the rat. *Transplantation,* **38,** 3.

PEACOCK J.H. & TERBLANCHE J. (1967) Orthotopic homotransplantation of the liver in the pig. In: *The Liver* (ed. A. E. Read), p. 333. Butterworths, London.

ROSER B.J. & FORD W.L. (1972) Prolonged lymphocytopenia in the rat. *Aust. J. exp. Biol. Med. Sci.* **50,** 185.

ROSER B.J., KAMADA N., ZIMMERMANN F. & DAVIES H.FF.S., (1983) Immunosuppressive effect of experimental liver allografts. In: *Liver Transplantation* (ed. R. Y. Calne), p. 35. Grune and Stratton, New York.

STUART F.P., WEISS A. & FITCH F.W. (1979) Induction and maintenance of immunological enhancement. In *Immunological Tolerance and Enhancement* (eds F. P. Stuart and F. W. Fitch), p. 29. MTP Press, Lancaster.