The Immunosuppressive Agent 15-Deoxyspergualin Induces Tolerance and Modulates MHC-Antigen Expression and Interleukin-1 Production in the Early Phase of Rat Allograft Responses

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THE new immunosuppressant 15-deoxyspergualin (DOS) is approximately as effective as cyclosporin A at prolonging allograft acceptance, but appears to act by a different mechanism. The aim of this study was to gain more insight into the possible mechanism(s) of DOS. The methods used for this purpose included kidney and skin grafting, immunoperoxidase histology, FACS analysis, and tests of interleukin-1 (IL-1) production.

RESULTS

MHC fully allogeneic rat kidney grafts (Dark Agouti to Lewis) are accepted indefinitely when DOS (2.5 mg/kg/d IP) is applied from day 0 to day +13. DOS induces specific, immunological tolerance, as shown by indefinitely accepted donor type and normally rejected third-party skin grafts on day +150.

As can be shown with MHC allogeneic kidney as well as skin grafts (normal survival periods, 7 to 9 days), DOS is highly effective at prolonging graft survival (kidneys, >250 days; skin, 23 to 27 days), when treatment begins between days +1 and +3, but not between day +4 and +6.

Frozen tissue sections of surviving kidneys (treatment beginning on day +1, +2, or +3) show a remarkable downregulation of MHC class I (MRC-OX18) antigens, but not class II (MRC-OX6) antigens on various kidney cells (see Table 1). However, when treatment begins as late as day +5, DOS fails to inhibit upregulation of class I antigens as shown by rejected kidneys.

FACS analysis of LEW rats treated with DOS in vivo (2.5 mg/kg IP for 7 days) indicated that class I antigens are downregulated on splenic macrophages, but class II antigens are not. This effect is dose dependent (2.5 to <5.0 mg/kg). In contrast, the expression of class I antigens on B and T lymphocytes is unaltered. Furthermore, DOS induces the appearance of a second population of MRC-OX41+ peritoneal exudate macrophages as early as 24 hours after treatment begins. This population, which is significantly poorer in MHC class I expression, reaches its maximum number of cells on the seventh day of treatment and disappears within 14 days after the last DOS application.

IL-1 production by splenic and peritoneal exudate macrophages treated in vitro with DOS is reduced significantly at lower DOS dosages (0.005-0.5 mg DOS/mL culture

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Table 1. Influence of Timing of DOS Application on MHC Classes I and II Antigen Expression in Normal Rat Kidneys and Kidney Allografts in the DA to LEW Strain Combination

Strain Combination	Investigated Organ*	DOS† (mg/kg)	Treatment Begins	Fate of Graft	Antigen Expression: MHC Class I TEC	MHC Class I GC‡	MHC Class I DC	MHC Class II TEC	MHC Class II DC
—	LEW kidney	—	—	–	++	+	++	+++	++++
—	DA kidney	—	—	–	++	+	++	+++	++++
DA→LEW	DA kidney	—	—	Rejected	++++	+++	+++	++++	+++
DA→LEW	DA kidney	2.5	Day +1	Accepted	–	–	–	+++	+++
DA→LEW	DA kidney	2.5	Day +3	Accepted	–	–	–	++	+++
DA→LEW	DA kidney	2.5	Day +4	Rejected	+/–	+/–	+/–	++++/++	+++
DA→LEW	DA kidney	2.5	Day +5	Rejected	++++/++	+	++	++++	+++
DA→LEW	DA kidney	5.0	Day +5	Rejected	++++/++	+	++	++++	+++

*Conventional immunoperoxidase histology of frozen donor kidney tissue sections on day +6 postoperatively.
†DOS treatment: daily IP injections; monoclonal antibodies for MHC staining: MRC-OX18 for class I and MRC-OX6 for class II. *Abbreviations:* DOS, 15-deoxyspergualin, TEC, tubular epithelial cells; GC, glomerular cells; DC, dendritic cells. –, no detectable reactivity; +, very weak; ++, weak; +++, strong; ++++, very strong reactivity.
‡GC are throughout negative in class II.

medium), as was shown in a direct IL-1 assay using rat thymocytes as target cells.

CONCLUSIONS

1. Short-term DOS treatment may initiate long-standing specific tolerance, which even extends to skin grafts.
2. DOS appears to interfere with antigen-presenting cells during the induction phase of allograft responses.
3. DOS appears to reduce the expression of graft MHC class I antigens, ie, of major target antigens, when given within the first days after transplantation.