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Mechanisms Underlying the Development of T-Cell Tolerance Following Interruption of Signalling at the CD28/B7 and CD40/gp39 Interface

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WE HAVE SHOWN previously that combined but not discrete transient perioperative blockade of CD28/B7 and CD40/gp39 costimulatory pathways results in indefinite prolongation of islet allograft survival.[1] Similar observations have also been made in the realm of solid organ transplantation (Tx).[2] Additionally, the use of both rhCTLA4-Ig and anti-gp39 mAb (but not alone) have also resulted in abrogation of morphologic changes considered pathognomonic of posttransplant vasculopathy.[3] Although the profound immunosuppressive effects of contemporaneous blockade of both costimulatory pathways have been established, not evident, however, are the underlying cellular mechanism(s) responsible for the observed outcome. The present study was therefore designed to address the latter issue.

MATERIALS AND METHODS

Animals

Inbred male Mls-disparate DBA/2 (H-2^d; MIS^d; $V\beta6^-$ TcR) and C3H (H-2^k; Mls^c; $V\beta^{6+}$ TcR) mice were used as donors and recipients of islet cell Tx, respectively. The recipients were rendered diabetic by previous (day − 4) treatment with streptozotocin (300 mg/kg IV).

Recipient Treatment and Follow-Up

Subsequent to islet cell Tx, the animals were treated with either rhCTLA4-Ig (group I; 200 μg on days 0, 2, 4, and 6 post-Tx IM) or anti-gp39 mAb (group II; 250 μg on days 0, 2, and 4 post-Tx IP). Additionally, in a distinct set of animals, these two molecules were also used contemporaneously (group III), as were isotype-matched irrelevant control antibodies (group IV). In animals with long-term euglycemia, the presence of donor-specific tolerance (DST) was ascertained by transplanting donor and third-party skin allografts. In the latter group of animals, in vitro analysis (flow cytometry, one-way MLR, suppressor cell, and TcR cross-linking assays) were also performed.

RESULTS AND DISCUSSION

As has been documented previously,[1] animals in groups I, II, and IV had comparable graft survivals with recurrence of hyperglycemia (in the majority of recipients) within 26 days post-islet Tx. On the contrary, indefinite (>180 days) islet allograft survival was witnessed in all animals in group III. These animals also had evidence of stable DST, for they rejected third-party but not donor skin allografts when challenged on ~d120 post-islet Tx. The deletion of autoreactive $V\beta6^+$ TcR-bearing T cells in DBA/2 but not C3H mice during ontogeny provided a tool that was used as a surrogate marker to delineate the cellular mechanisms underlying the observed outcome. Interestingly, while no peripheral deletion of $V\beta6^+$ $CD4^+$ T cells was evident in animals in group III, there was marked reduction in their proliferative response when exposed to anti-Vβ6 mAb in a TcR cross-linking assay. This latter observation suggested that clonal anergy but not deletion is perhaps the predominant mechanism responsible for indefinite islet and challenge skin allograft survival witnessed in recipients treated with a short contemporaneous perioperative course of rhCTLA4-Ig and anti-gp39 mAb. The long-term stability of the observed DST in these animals is currently being investigated.

REFERENCES

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0041-1345/99/$–see front matter
PII S0041-1345(98)02067-3