Transpl Int (2000) 13 [Suppl 1]: S474–S475

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Allo-tolerance and allorejection responses ex vivo

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Abstract Spleen cells from fully immune competent mice show different intracellular STAT responses to alloantigen. Cells from mice primed to accept the alloantigen have low STAT 6 and fragmented STAT 4, compared to cells from mice primed to reject the same alloantigen.

Key words STATs · Splenic lymphocytes · Allotolerance · Allorejection

Introduction

T lymphocytes may respond to alloantigen aggressively or suppressively, resulting in allorejection or allotolerance, respectively. We have attempted to identify some of the intracellular events which guide these alternative responses. In vitro data from TH1 or TH2 cell lines suggest that differential signalling via STATs (*s*ignal *t*ransducers and *a*ctivators of *t*ranscription) correlate with T cell phenotype [1]: interferon γ activates STAT 1, a TH1-type mediator, and IL4 activates STAT 6, a TH2-type mediator. Here we ask if a similar correlation occurs in an ex vivo model derived from normal mice.

Materials and methods

Fully immune competent CBA mice were rendered tolerant to BALB/c heart graft alloantigens by antibody-mediated CD4 + CD8 blockade [2]. Spleen cells from the CBA $^{(BALB/c\text{-}tolerant)}$ mice were compared to CBA $^{(BALB/c\text{-}rejected)}$ mice, where the latter had previously rejected a BALB/c skin graft. In each case, the spleen cells were harvested and challenged ex vivo by 5 days coculture with irradiated allo-antigenic (BALB/c) target cells. The cells were then lysed and probed for STATs by western blot.

Results

Splenic lymphocytes from CBA $^{(BALB/c\text{-}tolerant)}$ and CBA $^{(BALB/c\text{-}rejected)}$ mice showed the pattern of STAT induction in Table 1.

Table 1 Pattern of STAT induction

	BALB/c-tolerant	BALB/c-rejected
STAT 1 (ca 90 kDa)	++++	++++
STAT 4 (ca 90 kDa)	++[a]	++
STAT 5 (ca 90 kDa)	+	+
STAT 6 (ca 110 kDa)	+	+++

[a] Plus novel ca 40-kDa fragment

Control cultures of normal CBA spleen cells grown in either growth medium alone, or supernatant from the allotolerant, or allorejected cultures, were used to determine background levels of each STAT protein. These controls also showed that there was no "bystander" STAT induction by cytokines present in the different culture combinations. By comparing secondary responses to alloantigen, we avoided variables due to response kinetics.

Discussion

In experiments ex vivo, we have used spleen cells already primed to either accept or reject BALB/c alloantigen. By comparing STAT induction in response to alloantigen we found that allotolerance was associated with proteolysis of STAT 4; these cells also showed suppression of STAT 6 induction compared to the cells from allo-rejected mice. This suggests that the TH1:TH2 paradigm, wherein TH1 type is associated with rejection and TH2 type with tolerance, does not apply ex vivo. In contrast, the intracellular signals which regulate alloresponsiveness are more complex and appear to include differential processing of induced STAT 4 and differential expression of STAT 6.

References

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