Isolation and Characterisation of the Immunosuppressive Peptides in the Rat Testis

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Abstract

The rodent testis is recognised as an immune-privileged site in which allogeneic tissue grafts can survive for long periods of time, possibly indefinitely. Theories developed to date suggest that the testis contains specific immunosuppressive factors that inhibit lymphocyte activation in this site (for review see Maddocks and Satchell, 1990, Streilein, 1993, Filippini et al., 2001). However the nature of these factors has not been well characterised to date.

The present study was conducted in an attempt to isolate and characterise the immunosuppressive factors in the rat testis, and to determine the probable mechanism by which immunosuppression is achieved. A crude testicular extract was used as the source material for the investigations described.

Immunosuppressive activities were assessed using a Con A-induced splenic T cell proliferation bioassay. The splenic T cells were isolated using a percoll density gradient separation which resulted in T cells expressing mainly c/βT cell receptors as determined by FACS analysis. Crude testicular extract suppressed mitogen (Con A or PHA) induced splenic T-cell proliferation. Removal of significant amounts of steroid from testicular homogenate by dextran-charcoal extraction did not abolish the immunosuppressive activities. Separation of this crude testicular extract using a Sephadex G 25 PD-10 column resulted in three molecular weight fractions: Mr > 5, Mr 1-5 and Mr < 1 kDa. The immunosuppressive activity was observed in fractions of Mr > 1
kDa, with the strongest immunosuppressive activity present in the Mr 1-5 kDa fraction. Using a Superdex Peptide PC 3.2/30 column, the strongest immunosuppressive activity was found to have a molecular weight of around 3.5 kDa.

This result contrasts with previous reports of similar investigations on rodent testis immunosuppressive activities, and attempts to further purify these immunosuppressive factors were focused on the low molecular weight substances. The semi-purified (low molecular weight) immunosuppressive activities were found to be relatively heat and pH stable, but were sensitive to trypsin, suggesting they were most likely peptidic in nature.

Further analysis involved ion-exchange chromatography, high performance liquid chromatography (HPLC) and reversed phase high performance liquid chromatography (RP-HPLC) methods. The use of RP-HPLC employed a µRPC C2/C18 column with either trifluoroacetic acid (TFA, 0.1%, v/v) or heptfluorobutyric acid (HFBA, 1%, v/v) and acetonitrile (0-80% v/v) as the elution buffer. This resulted in reduced immunosuppressive activity. Similar results were also observed with the high molecular weight fractions and such effects have also been reported by others working in this area (Saxena et al., 1988). The results suggested that the immunosuppressive factors are relatively unstable under the purification conditions employed and may be oligomeric in nature.

Since the proliferation of activated T cells is related to the production of IL-2 and the expression of the IL-2-receptor, investigations were undertaken on the
production of IL-2 by activated T cells cultured in the presence of testicular immunosuppressive factors. The results show that both the crude and high molecular weight, but not low molecular weight immunosuppressive factors inhibited IL-2 production. In this context, at least two immunosuppressive mechanisms are present in the testis extracts. The high molecular weight factors suppress the proliferation of activated T cells via the inhibition of IL-2 secretion, which has also been reported previously by Pöllänen et al., (1990) and attributed to a TGF-β-like protein named 'protectin' (Pöllänen et al., 1988).

However inhibition of activated T cell proliferation by low molecular weight (LMW-TE) factors did not affect IL-2 production, and was shown in the present study to induce specific programmed cell death (apoptosis) which was not due to general cytotoxic effects.

Although the TE immunosuppressive peptides suppressed both CD4+ and CD8+ T cell subsets, the strongest suppression was found to be extended to the CD4+ T cell subset. It is possible that LMW-TE could possibly be involved in the down regulation of CD4+ T cell populations in the testis as it has been previously reported that in the rat testis CD8+ T cells are more frequently observed compared to CD4+ T cells (Hedger et al., 1998b, Tompkins et al., 1999).

From the present study it can be concluded that the mechanisms regulating the status of the testis as an immunologically privileged site involve complex, multiple and possibly redundant mechanisms to both inhibit an autoimmune
attack on the haploid germ line on the one hand and yet to also allow immunologic responses against pathological events to take place on the other. The presence of high and low molecular weight peptides in the rat testis as reported in the present study contribute to these events directly or indirectly. However, the specific nature of the various components of the regulatory pathways that maintain the unique environment of the testis are still to be elucidated.
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