Early pregnancy factor, chaperonin 10 and rheumatoid arthritis; the story unravels

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Treatment with recombinant chaperonin 10 (rCpn10) was shown to be efficacious in rheumatoid arthritis patients. Chaperonin 10 (Cpn10) is believed to represent the immunosuppressive Early Pregnancy Factor (EPF) previously identified in pregnancy serum. Herein we argue that rCpn10’s immunosuppressive activity was likely due to endotoxin tolerance. Furthermore, Cpn10 is not elevated in pregnancy serum, making it a poor candidate for EPF. Although the Cpn10/EPF story provided an enticing rationale, the evidence that Cpn10 is EPF, and that Cpn10 has anti-inflammatory activity is far from compelling.

Early pregnancy factor (EPF) was originally described in a 1977 Lancet paper as an immunosuppressive factor present in human pregnancy serum that could be detected by a rosette inhibition test (RIT) [1]. Lymphocytes form spontaneous rosettes with sheep red blood cells via CD2, an activity that can be inhibited by anti-lymphocyte anti-serum or anti-CD2 antibodies [2]. Incubation of lymphocytes with pregnancy serum (but not normal serum) significantly reduced the concentration of anti-lymphocyte anti-serum required to inhibit rosette formation [1]. EPF might more accurately be described as a RIT activity, as no actual factor was identified in this study [1]. The activity was identified in pregnancy serum from other mammals, including cows, horses, pigs and mice, albeit with consistency issues [3]. In humans RIT activity was present until week 28 of pregnancy, but was back to baseline at 31 weeks [4-6]. Using the RIT assay, EPF was subsequently professed to be heat shock protein 10 (Hsp10), also known as chaperonin 10 (Cpn10) [7,8].

Buoyed by the observation that rheumatoid arthritis is often ameliorated during pregnancy [9], recombinant Cpn10 (rCpn10) was subsequently developed by CBio Ltd. as an immunomodulatory biologic (XToll) [10]. A randomised, double-blind, multi-centre human trial supported the view that rCpn10 (5-10 mg intravenously twice a week) was efficacious for treating symptoms of rheumatoid arthritis (RA). The trial results were published in Lancet [11]. Efficacy was subsequently reported for rCpn10 in the treatment of multiple sclerosis [12] and psoriasis [13], with a trial for lupus also undertaken (NIH USA ClinicalTrials NCT01838694).

However, after publication of the RA trial results [11], some reservations were raised. A comment published in Lancet argued that endotoxin tolerance may have played a role; rCpn10 was produced in bacteria and may thus have been contaminated with low levels of endotoxin [14]. Endotoxin tolerance is a well described phenomenon whereby repeated exposure to endotoxin results in anti-inflammatory/ immunosuppressive immunomodulation [15,16].

De-identified microarray, DAS28 and pharmacokinetic data from a small number of patients enrolled in the RA trial [11] was made available by Invion Ltd., who purchased CBio and are not pursuing development of XToll. A mean Cmax for rCpn10 of 1820 ± 115 ng/ml and T1/2, of 1 ± 0.11 hour was determined in a subgroup of patients (n=3) enrolled in the RA trial. PBMC were obtained at baseline (T0) and after 56 days of intravenous treatment with 10 mg of rCpn10 twice a week (T56). Paired (T0/T56) microarray data from PBMC from 4 patients (GSE112809) passed quality control assessments. The DAS28 score improvement in each of these 4 patients over this period (5.67 to 2.81, 5.58 to 4.4, 4.83 to 3.54, and 6.3 to 5.38; paired t test p=0.048) was consistent with [11]. Differentially expressed genes (DEGs) were identified using a paired comparison for T56 versus T0 for each of the 4 patients. The analysis identified 328 DEGs that were up-regulated and 139 DEGs that were down-regulated at T56 (using a p<0.05 cut-off) (Supplementary File). Using Gene Set Enrichment Analyses (GSEA), the up-regulated DEGs were compared with publicly available transcriptional profiles from (i) human peripheral blood treated with LPS [17] and (ii) human peripheral blood treated with monophosphoryl lipid A (MPLA) [17], which is a Toll-like receptor 4 (like LPS) and a Toll-like receptor 2 agonist. Significant enrichments were observed (i) LPS, NES=1.31, FDR=0.037 and (ii) MPLA, NES=1.43, FDR=0.0038 (Figure 1a). Analysis of down-regulated genes using Ingenuity upstream regulator analysis identified the immunosuppressive cytokines IL-10 (p=0.043) and TGFβ1 (p=0.045) as potentially responsible for down-regulation of several genes (Figure 1b), consistent with [11], the DAS28 score improvements, and also endotoxin tolerance [16]. As patient numbers were low and low stringency cut-offs were used, these results might be viewed as indicative only, but they are consistent with induction of endotoxin tolerance by rCpn10 (as suggested [14]). Endotoxin contamination of recombinant proteins is hardly new, although bioassays or murine systems that reliably predict endotoxin tolerance (e.g. from repeated low dose endotoxin exposure) are arguably not well developed [18,19]. Humans are as much as 100,000 fold more sensitive to LPS than mice [20].

The molecular basis for RIT activity in pregnancy sera is unclear, as is the selectivity and specificity of the RIT assay. We thus surveyed the proteomics literature to ascertain whether Cpn10 (or Hsp10) was actually present in human pregnancy serum. In 6 studies [21-25] only one study identified one Hsp10 peptide [26], although 100-800 other proteins were identified in these studies. Hsp10 is not intrinsically
1. Morton H, Rolfe B, Clunie GJ (1977) An early pregnancy factor detected in human pregnancy sera. Sera obtained from pregnant Balb/c mice and non pregnant mice (cont) at the indicated times was analysed for Cpn10 concentration in duplicate using a capture ELISA.

Figure 1. PBMC-derived RNA from 4 rheumatoid arthritis patients treated with rCpn10 was subjected to gene expression microarray analysis using the Affymetrix Human Genome U133 Plus 2.0 Array platform. Microarray data was converted into expression measures and normalised with robust multiarray average (RMA) and gene ID conversion by affy Biocductor package. The quality control was performed using arrayqualitymetrics Biocductor package. The paired t-test based on the limma Biocductor package was used to identify differentially expressed genes (DEGs) between T0 (before rCpn10 treatment initiation) and T36 (after 56-days of rCpn10 treatments) (p < 0.05 and fold change > 1.0). (a) Up-regulated DEGs (after rCpn10 treatment) (n=328) were used in pre-Ranked Gene Set Enrichment Analyses. These were performed using fGSEA (R) and pre-ranked gene expression fold change data from microarrays of human blood treated for 90 mins with LPS or MPLA (GSE72557). Of the 328 rCpn10 up-regulated genes, 295 were present in the two data sets. Of these, 102 (32+38+32) were identified as core enriched genes by fGSEA for LPS and/or MPLA stimulation. (b) Down-regulated genes (after rCpn10 treatment) were analysed by Ingenuity Pathway Analysis (IPA) upstream regulator function (direct and indirect). A small but significant IL-10 and TGFβ1 signature was identified (numbers of “Target molecules in dataset” from IPA output are indicated).

In summary, the evidence that Cpn10 is EPF, and that Cpn10 represents a distinctive state of alternative polarization (M2) in human mononuclear cells. The quality control was performed using arrayqualitymetrics Biocductor package. The paired t-test based on the limma Biocductor package was used to identify differentially expressed genes (DEGs) between T0 (before rCpn10 treatment initiation) and T36 (after 56-days of rCpn10 treatments) (p < 0.05 and fold change > 1.0). (a) Up-regulated DEGs (after rCpn10 treatment) (n=328) were used in pre-Ranked Gene Set Enrichment Analyses. These were performed using fGSEA (R) and pre-ranked gene expression fold change data from microarrays of human blood treated for 90 mins with LPS or MPLA (GSE72557). Of the 328 rCpn10 up-regulated genes, 295 were present in the two data sets. Of these, 102 (32+38+32) were identified as core enriched genes by fGSEA for LPS and/or MPLA stimulation. (b) Down-regulated genes (after rCpn10 treatment) were analysed by Ingenuity Pathway Analysis (IPA) upstream regulator function (direct and indirect). A small but significant IL-10 and TGFβ1 signature was identified (numbers of “Target molecules in dataset” from IPA output are indicated).

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