

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Cooper HL, Louafi F, Friedmann PS. A case of conjugal azathioprine-induced contact hypersensitivity. *N Engl J Med* 2008;359:1524-6.

## **2 phase IFN- $\gamma$ Elispot**

The husband's peripheral blood mononuclear cells (PBMC, mainly monocytes and lymphocytes) were isolated by centrifugation over a density gradient (lymphoprep, Axis-Shield Norway). Cells were plated at 1.5 million cells/ml/well of a 24-well plate in serum-free RPMI 1640 media (GIBCO) for 1 hour to allow the monocytes to adhere. The supernatant was then gently pipetted off (mainly lymphocytes), and saved in an adjacent well. 1ml of RPMI media containing 5% human blood group AB serum and 0, 0.1, 1, 10 or 100 $\mu$ M azathioprine or 6 mercaptopurine were added to the adherent monocytes. After 24 hours the drug-containing medium was removed gently to leave the adherent, drug-bathed monocytes in the well; the lymphocytes in the adjacent wells were agitated and pipetted back onto the monocytes.

Elispot Multiscreen<sup>TM</sup>-IP 96-well plates (Millipore, UK) were coated overnight at 4°C with 10  $\mu$ g/ml of anti-IFN- $\gamma$  monoclonal antibody in PBS (mAb 1-D1K, Mabtech, UK). The plates were then blocked in 10% FCS containing RPMI for 90 minutes and washed 3 times with PBS. PBMC from the two-phase assay above plated in RPMI 1640 medium (GIBCO) containing 5% human AB serum (Sigma, UK) were transferred at a density of  $2 \times 10^5$  cells per well. *Candida albicans* crude extract at a 1/150 dilution was used as a positive control. The cells were then cultured in an incubator at 37°C for 96 hours.

Subsequently, the plates were washed once with water and 4 times with PBS before the addition of biotin-conjugated anti-IFN- $\gamma$  monoclonal detection antibody (mAb 7-B6-1, Mabtech, UK) in 1% human AB serum PBS. After 90 minutes the plates were washed 5 times with PBS and streptavidin-HRP conjugate (Sigma, UK) was added at a 1:1000 dilution in PBS for 1 h at 37°C. Afterwards, plates were washed 5 times with PBS. The spots were then revealed using a solution of 100 $\mu$ l of NBT/BCIP (Invitrogen, UK). The reaction was stopped by addition of water after 15 minutes, and the plates were left to dry. Spot count and analysis was performed using an Elispot reader and AID Elispot Reader software. Results are expressed as spot-forming cells per million cells after subtracting the background.