Supplementary data, S1

Methods

Cloning of the recombinant fusion proteins

The gen coding for the RD (human IL-15Rαsushi domain + subsequent 12 amino acids) (amino acids 31-107) fused by a $G_3SG_4SG_3SG_4SLQ$ linker to human IL-15 was synthesised synthetically (GENEART, Regensburg, Germany) and a BglII and a NotI restriction site was added at the N- and C- terminus, respectively. By PCR the IL-15 (5'-GGAAGATCTGGATCTGGCGGAGGAGGAAGCTTACAG-3'; 5'-TAGAAGGCACAGTCGAGG-3') and RD (5'-TAATACGACTCACTATAGG-3'; 5'-CATCAGCGGCCTGCACCTCCCGCGGCCGCTAAACTAT-3') fragment were amplified or in the case of RD_IL-15 directly cut and cloned in the pAB1 vector (BglII/NotI). In a second cloning step the scFv36 was amplified by PCR (5'-CTCGCGGCCCAGCCGGCCATGGCCCAGGTGCAGCTGGTCGAGTCTGGC-3'; 5'-ACCAAGCTGGAAATCAAGAGATCTTCC-3') and inserted (SfiI/ BglII) N-terminally of the RD or rather IL-15 fragment, generating the fusion genes scFv_IL-15, scFv_RD and scFv_RD_IL-15, respectively. Finally, the constructs were cloned (SfiI/ NotI) into the eukaryotic expression vector pSecTagAHis (modified pSecTagA (Invitrogen, Karlsruhe, Germany) lacking the Myc-tag), which provides an Igk leader and a Hexahistidine-tag. In addition, the construct RD_IL-15 was also cloned (SfiI/NotI) into pSecTagAHis.

Expression and purification of the fusion proteins

Stably transfected cell lines expressing the fusion proteins were generated by transfecting corresponding plasmid-DNA with Lipofectamine2000 (Invitrogen, Karlsruhe, Germany) into HEK293 cells. Stable transfectants were selected in the presence of zeocin (300 μg/ml) (Invitrogen, Karlsruhe, Germany). In the case of scFv_RD_IL-15 and scFv_IL-15 producer

cells, single clone dilutions were performed, in order to raise the production yield. Cells were expanded and grown to 90% confluence in RPMI 5% FBS before switching to serum free Opti-MEM I medium (Invitrogen, Karlsruhe, Germany). Opti-MEM I was replaced every 3 days for 3-4 times. Supernatants were pooled and proteins were concentrated by ammonium sulphate precipitation (60% saturation), before loading onto a nickel nitrilotriacetic acid column (Qiagen, Hilden, Germany) previously equilibrated with phosphate-buffered saline (PBS). After a washing step with 50 mM sodium phosphate buffer, pH 7.5, 250 mM NaCl and 20 mM imidazole, the recombinant fusion proteins were eluted with 50 mM sodium phosphate buffer, pH 7.5, 250 mM NaCl and 250 mM imidazole. Protein fractions were pooled and dialyzed against PBS.