Effector and Regulatory T lymphocytes

The Immune System has to distinguish normal body components, to which it must remain tolerant, from invaders such as bacteria and viruses, and abnormal tissues such as cancer, to which it must react and eliminate. Failure of tolerance to self causes ***autoimmune diseases***, such as ***diabetes*** and ***multiple sclerosis***, whereas we would like to induce tolerance to donor organ grafts to avoid long term immunosuppressive drugs.

The elimination of abnormal and invading organisms is performed and controlled by **effector T lymphocytes**, while tolerance is maintained by **regulatory T cells**. An infected or **dying** cell in the body is taken up and digested by the specialised dendritic cell that displays peptide fragments from the **dying** cell to the T lymphocytes. If the **effector T lymphocytes** predominate an aggressive immune response occurrs, but if **regulatory T cells** are present in sufficient numbers they will limit any aggression and enforce tolerance.

We aim to identify genes specific to the **regulatory T cells** that can be used to develop diagnostic tests for surrogate biomarkers for tolerance during the treatment of autoimmunity or graft rejection. This will allow the use of lower and less toxic doses of immunosuppressive treatment and the rational development and clinical testing of new, ***tolerance inducing therapies***.

Gene Discovery by SAGE

Once we have separated the **effector**, **regulatory** and **dendritic** cells by cloning them in tissue culture, we can use a method known as SAGE (Serial Analysis of Gene Expression) to identify genes that are turned on specifically in the **regulatory T cells** but not in the effector T lymphocytes.

Each genes that is turned on makes a unique mRNA gene transcript, identified by the sequence of nucleotides, and ending in "polyA". An **E**nzyme can be used to cut each mRNA molecule into smaller fragments, and the 10 nucleotide **tag** (plus the 4 nucleotides of the **E**nzyme site) nearest the polyA tail can be extracted. Each **tag** has a sequence, that together with the positional information (**E**nzyme site), is sufficient to identify which mRNA, and therefore which gene, it came from. The **tags** are concatenated into long, linear pieces of DNA that can be serially sequenced using fluorescent dye terminator technology and an automated capillary gel electrophoresis machine.

The SAGE computer software takes the Sequence Traces from the machine and identifies how many times each unique **tag** appears, and uses the **tag** sequence to look up the corresponding gene in the appropriate genome database. The number of times the **tag** for any given gene was found in the concatamers from each cell type is directly proportional to the mRNA levels found in that cell. The vast majority of genes (perhaps 30,000) will be expressed at similar levels in both effector T lymphocytes and **regulatory T cells**, but computerised subtraction can identify those which are candidates as ***regulatory specific*** genes.