328
Comparative evolutionary analysis of IL6 in lagomorphs

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Background and aims: Interleukin 6 (IL6), also known as interferon beta 2, is a class-I helical cytokine with a broad spectrum of biological activities in humoral and cellular defense. This class of cytokines has a gene structure conserved throughout vertebrates, with five coding exons.

IL6 is involved in the immune response against rabbit hemorrhagic disease virus that causes a highly fatal disease in the European rabbit. Previously, IL6 from European rabbit samples belonging to the subspecies *Oryctolagus cuniculus cuniculus,* was shown to differ from the other mammals by extending for further 27 amino acids. This difference results from a mutation in the typical stop codon into a glutamate encoding codon. However, in other leporids (*Sylvilagus* spp. and *Lepus* spp.) that diverged from European rabbit approximately 12 million years ago this mutation was also not present.

The purpose of this study was to confirm the mutation of the stop codon in other lagomorph specimens: *Oryctolagus cuniculus algirus, Brachylagus idahoensis, Sylvilagus* bachmanii, *Lepus europaeus* and *Ochotona princeps.*

Methods: The IL6 gene was PCR-amplified and sequenced for the five lagomorph species. The obtained sequences were translated and compared with other mammalian IL6 sequences retrieved from public databases (GenBank, Ensembl and Uniprot).

A maximum-likelihood (ML) tree was inferred in MEGA5 with the following options: HKY+G model, 500 bootstrap replicates and partial deletion to gaps/missing data treatment.

Results: We confirmed the presence of the mutated stop codon in both *O. c. cuniculus* and *O. c. algirus*. In agreement with previous reports, we found that the stop codon is not mutated in *S. bachmanii* and *L. europaeus*. We further extended this observation to the leporid *B. idahoensis* and ochotonid *O. princeps.*

In rabbits, sequence translation of IL6 continues into the exonic sequence and stops in the next STOP codon (81 nucleotides downstream). Typically, the IL6 protein has five cysteine residues that might be important to establish disulfide bonds. In rabbit, the 27 amino acid extension has four more cysteine residues.

The inferred phylogeny for the IL6 gene is in agreement with what has been accepted for the mammals and lagomorphs.

Conclusions: Our results indicate that in the ancestral of the *Oryctolagus* genus, (approximately 2 million years ago), a single mutation at exon 5 occurred that made IL6 longer than for the other mammals. Biological implications of this extension remain to be assessed but the occurrence of the 4 extra cysteine residues might suggest some functional relevance.

Myb-independent macrophages: a family of cells that develops with their tissue of residence and is involved in its homeostasis

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Background and aims: In most metazoan, all tissues contain phagocytes 'in residence', generally termed 'macrophages' in vertebrates. In contrast to myeloid cells produced continuously by the bone marrow, tissue resident macrophages develop during embryogenesis together with their tissue of residence, and persist in adulthood, independently of hematopoietic stem cells and the transcription factor *Myb*. They therefore represent an independent lineage from blood monocytes, dendritic cells, and monocyte/macrophages that are recruited to tissues during inflammation.

Methods: We reinvestigated the origin and developmental relationship between different tissue resident myeloid populations using complementary fate mapping strategies based on Cre/Lox systems. Fate mapping of HSC-derived cells was performed sing Flt3-Cre mice whereas HSC- and Myb-independent macrophages were pulse labeled during embryonic development using tamoxifen in Csf1r-mer iCre mer mice.

Results and conclusions: Tissue resident macrophages functions are yet to be completely defined. They all share the ability to scavenge toxic compounds, lipids, microorganisms, dead cells and contribute to tissue remodeling, via phagocytosis and the production of growth factors. In contrast the production of inflammatory mediators seems more associated with bone marrow derived cells. Tissue resident macrophages and bone marrow derived myeloid cells thus differ in developmental origin and functions. A genetic and molecular dissection of resident macrophages functions will reveal their roles in tissue metabolism and the maintenance of homeostasis independently of the extravasation of inflammatory leucocytes, and in the control of the recruitment of bone marrow derived cells in overt inflammation.

511
Immune escape strategies of a recently emerged contagious cancer, Devil Facial Tumour Disease

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Background and aims: In rare cases cancer cells do not die within a single individual, but successfully pass between individuals becoming a contagious cancer derived from a single neoplastic cell. Devil Facial Tumour Disease (DFTD) is one such contagious cancer that has emerged in the Tasmanian devil, a carnivorous marsupial endemic to the island of Tasmania. Despite an efficient immune system DFTD does not elicit a protective immune response from host devils, resulting in 100% mortality of affected animals and rapid decline of the species. The emergence of DFTD provides an opportunity to understand the immunological basis for the transmission of a tumour in a wild population.

Results: We have shown that DFTD cells down-regulate cell surface MHC class I molecules both *in vitro* and *in vivo*. The loss of class I molecules is not due to structural mutations, but to epigenetic down-regulation of genes essential for antigen processing, including β_2-microglobulin (β_2m), the Transporters associated with Antigen

