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Thesis Abstract: Investigation of the Therapeutic Potential of Transgenic CD40 Ligand Expression

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The CD40 ligand (CD40L) molecule is central to innate and adaptive immunity. CD40L expression is very tightly regulated whereas its CD40 receptor is constitutively expressed by many different cell types. CD40L is expressed transiently on helper T cells (Th) only after activation by specific immune recognition molecules carried by professional antigen presenting cells, in particular, dendritic cells (DC). CD40L subsequently binds to CD40 on DC to enable full Th activation. CD40 ligated DC produce interleukin-12 (IL-12) and contribute both to the development of IFNγ-secreting natural killer cells, a vital component of innate immunity, and of IFNγ-secreting type 1 Th (Th_1) cells. CD40 ligated DC also contribute to the development of IL-4- and IL-10-secreting Th_2 cells. CD40L on Th cells also binds CD40 on macrophages to enhance their cytotoxic functions. CD40L-expressing Th cells provide the 'help' pivotally required to activate other components of adaptive immunity responsible both for clearing invading pathogens and generating the memory cells required to prevent re-infection. Th-supplied CD40L binds (i) B cell CD40 to switch production of antibodies to more potent effector molecules that have higher avidity for antigen, and (ii) DC CD40 to prime then expand antigen-specific cytotoxic T lymphocytes (CTL). Activated NK cells and CTL are required both to eradicate malignant cells and cells infected with viruses or other intracellular pathogens.

Genetic CD40L deficiency causes the very rare HyperIgM Syndrome Type 1 (HIGM1), which is realistically modelled by genetically engineered CD40L-deficient mice. Neither CD40L-deficient patients nor mice make effective antibodies or mount cellular immune responses that would defend them against intracellular pathogens such as parasites. Consequently, the only potentially curative therapy is allogeneic stem cell transplantation or CD40L gene replacement. Here, we used a retroviral vector, which constitutively expressed CD40L, to genetically modify CD40L-deficient bone marrow cells, which were used to reconstitute partially the immunity of CD40L-deficient mice. The crucial importance of tight regulation of CD40L expression was revealed when these mice later developed lethal thymic T cell malignancy.

Growing tumours escape immune vigilance by genetic alterations that reduce their sensitivity to IFNγ. Using murine tumour models, we incorporated transgenic CD40L expression in therapeutic tumour vaccines to show that CD40L gene transfer augmented the immunogenicity of the host's tumour thus reducing its tumorigenicity. We translated this finding clinically to safety and immunogenicity testing of a transgenic CD40L- and IL-2-expressing leukaemia vaccine.

Finally, the common viral respiratory pathogen, respiratory syncytial virus (RSV) mainly infects young infants and the elderly to cause potentially lethal pneumonia. Both groups have reduced cellular and humoral immunity, which predisposes them to re-infection with RSV. Using a murine model, we showed first that simultaneous adenoviral expression of CD40L augmented primary RSV-specific Th_1 responses that were associated with accelerated pulmonary viral clearance. Second, we showed that expression of CD40L in RSV-F and RSV-G subunit DNA vaccines elevated antibody and cellular immune responses to RSV challenge four and eight months after the initial immunisation.

These results demonstrate the potent ability of CD40L gene transfer to solve the absolute immune deficiency caused by genetic lesions of CD40L. However, physiological regulation

of the transgene is required to prevent serious adverse consequences. In contrast, no adverse effects were observed after transgenic CD40L expression was used to overcome relative immune deficiencies imposed by malignancy and RSV infection.

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Thesis Abstract: Compound Specific Detection of Endogenous Steroid Abuse in Sport: A Metabonomic Perspective

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The complementary application of Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) were used in this study to provide compound specific detection of endogenous steroid misuse for improved anti-doping analysis. Administrations of synthetically derived dehydroepiandrosterone, androstenedione, 4-androstenediol, 5-androstenediol and testosterone were specifically confirmed on the basis of abnormal urinary excretions and low ^{13}C content of their respective diagnostic markers; 3α,5-cyclo-5α-androstane-6β-ol-17-one, 4OH-androstenedione, androst-2,4-diene-17-one/androst-3,5-diene-17-one, etiocholanolone sulfoconjugate and testosterone.

A comprehensive reference interval study established the natural variation, predominantly from diet, observed for $\delta^{13}C$ values in an elite athlete population (n = 1262) representing 13 countries. The minimum ^{13}C values recorded for the terminal androgen metabolites; androsterone and etiocholanolone were -25.3‰ and -25.8‰, respectively. A maximum of 3.8‰ was observed for the associated $\Delta\delta^{13}C$ value that incorporated 11keto-etiocholanolone as the endogenous reference compound. Parametric statistics were applied to these data sets to propose high confidence (mean ± 3 standard deviations) $\delta^{13}C$ and $\Delta\delta^{13}C$ limits of -27.0‰ and 4.0‰ to define endogenous steroid abuse. The use of pregnanediol as the endogenous reference compound provided a lower $\Delta\delta^{13}C$ limit of 3.0‰.

The combinatorial approach to GC-MS and GC-C-IRMS data analysis was used to investigate the potential for *metabonomics* – the study of a discrete metabolite set – to improve anti-doping science. This methodology allowed the simple interpretation of all relevant information concerning an individual's metabolism in order to make an informed decision with respect to a doping violation.

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