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Hypothesis Testing: CTLA4 Co-stimulatory Pathways Critical in the Pathogenesis of Human and Mouse Alopecia Areata

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To the editor:

Rodent models with autoimmune diseases provide many insights to unravel the complexities of human diseases as tools for hypothesis testing and preclinical drug efficacy screening (Sun *et al.*, 2008). Nearly ten years ago, we performed a comparative mouse and human gene array study to identify potentially dysregulated genes in alopecia areata (AA). One such gene was CLTA4, a co-stimulatory T cell ligand that binds B7.1 (CD80) and B7.2 (CD86) on antigen presenting cells (Carroll *et al.*, 2002). Since AA is an autoimmune disease in humans and mice, we hypothesized that CTLA4, through its co-stimulatory T cell and antigen presenting cell (APC) pathways, is a critical regulator of AA onset and maintenance based on studies in the mouse model of AA (Sundberg *et al.*, 1994). We performed pre-clinical studies by intraperitoneally injecting monoclonal antibodies against APC surface markers B7.1 (CD80) and B7.2 (CD86) and a monoclonal anti-CTLA4 antibody into C3H/HeJ mice with and without AA to disrupt T cell and APC interactions involving CTLA4. The studies conclusively showed that these monoclonal antibodies effectively prevented onset of AA in the mouse model (Carroll *et al.*, 2002; Sun *et al.*, 2008). Subsequently, we confirmed increased numbers of skin infiltrating cells and skin draining lymph node cells in AA mice expressed CD80/86 while CTLA4 was increased in both populations; primarily on $CD4^+/CD25^-$ cells (Zoller *et al.*, 2002). CTLA4 (CD152) was similarly found with significantly elevated expression in peripheral blood $CD4^+/CD25^-$ cells of AA patients (Zoller *et al.*, 2004a) and a significantly larger percentage of peripheral blood mononuclear cells expressed CD80 (Zoller *et al.*, 2004b).

Recently, two landmark papers were published, the first one, a human genome wide association study (Petukhova *et al.*, 2010), and more recently, another one that replicated

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CTLA4 as a major candidate gene for AA susceptibility in humans (John *et al.*, 2011), providing exciting data on human AA and validating the first *bona fide* susceptibility locus outside the HLA. The co-stimulatory locus containing the CTLA4 gene on mouse chromosome 1 (human chromosome 2) was not previously identified as a quantitative trait locus in the mouse AA model (Sundberg *et al.*, 2003; Sundberg *et al.*, 2004). However, the convergence of expression data from the AA model with the human studies confirm our hypothesis that antigen presentation in association with CTLA4 – CD80/86 co-stimulation is implicated in the development of AA and provides a crucial foundation for future human clinical studies. For example, the preclinical data from the mouse AA model could be used to support testing of Abatacept (Orencia®), a drug approved by the FDA to treat autoimmune rheumatoid arthritis (Moreland *et al.*, 2006), as a safe and effective therapy for human AA. Abatacept is a fusion protein with the extracellular domain of CTLA4 fused with the hinge CH2 and CH3 domains of human IgG1. It is a selective modulator that binds to the co-stimulatory protein CD28 on T cells and B7 proteins (CD80/CD86) on APCs. As a possible alternative, Ipilimumab (Yervoy®) is a fully humanized anti-CTLA4 monoclonal antibody FDA approved to treat metastatic melanoma; however, Ipilimumab has been documented to induce human autoimmune diseases in clinical use (Callahan *et al.*, 2010) while Abatacept has not so far. Abatacept is associated with an increased incidence of infections due to its immunosuppressive effects (Dubois and Cohen, 2009), but this may be acceptable to AA patients if it is shown to be efficacious for hair growth.

Why is there a delay in a detailed exploration of the CTLA4 pathway(s) in human AA? A lingering bias against animal models of human disease is widespread in the medical and lay community, partly based on the not uncommon lack of treatment efficacy confirmation and/or safety issues revealed in subsequent human clinical trials. The explosion of genetic analyses studies shows that at a cellular and genetic level there are many common regulatory pathways in human diseases and their respective animal models. When findings in a putative animal model for a specific human disease do not fulfill all the precise features of the human disease it most likely means that: 1) the original model selection was flawed or the human disease is incompletely or incorrectly characterized, 2) the human disease is heterogenic due to genetic, ethnic, age, and gender differences in the disease susceptibility and/or variations in environmental influence; issues that cannot always be replicated with inbred animal models in a regulated environment, or 3) the methodology in the model studies or subsequent human trials was flawed leading to systematic bias (van der Worp *et al.*, 2010). The challenge in developing any new therapy for human diseases involves the careful selection of a suitable preclinical model(s) to be used and well-defined human subjects to be evaluated. While such issues cannot be ignored, the identification of a relevant animal model for a human disease can prove invaluable for studying disease mechanisms and for developing and screening new treatments.

The discoveries made in humans, rats, and mice with AA over the past 20 years led to understanding just how complex its genetics, molecular mechanisms, and its molecular pathways are (Carroll *et al.*, 2002; Subramanya *et al.*, 2010) in regulating its susceptibility, progression, and resolution (John *et al.*, 2011; Petukhova *et al.*, 2010; Sundberg *et al.*, 2003; Sundberg *et al.*, 2004). Autoimmunity in humans and other mammals have many biological

and genetic features that share many common regulatory pathways and provide valid insights (Sundberg and Schofield, 2009). Though the AA models are not perfect representations of human AA, the fundamental concepts of AA in rodents and humans have proven remarkably similar thus far. By integrating findings from multiple sources, great advances will continue to assist in developing safe and effective treatments for human and animal diseases including AA.

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Abbreviations used

AA	alopecia areata
APC	antigen presenting cell
CD4, 8, 25, 80 (B7.1), 86 (B7.2)	CD4, 8, 25, 80 and 86 antigen
CTLA4 (CD152)	cytotoxic T-lymphocyte-associated protein 4
FDA	United States Food and Drug Administration
HLA	human leukocyte antigen
IgG1	immunoglobulin G1

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