# Murine Aortic Smooth Muscle Cells Fail to Present Protein Antigens in the Context of MHC Class II

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In atherosclerosis vascular smooth muscle cells (SMCs) participate in the formation of vascular tertiary lymphoid organs (Lötzer et al., 2010) and express class II major histocompatibility complex molecules (MHC II) (Hansson et al., 1986). The aim of the present study was to investigate the ability of SMCs to act as antigen presenting cells *in vitro.*

To this aim we employed the Ealpha (Eα)-GFP/Y-Ae system (Itano et al., 2003) that allows visualisation of antigen uptake, as the Eα is GFP labelled, and tracking of antigen presentation using the Y-Ae antibody to detects Eα when bound to MHC II.
Stimulation of murine primary aortic SMCs with IFN-γ (100 ng/mL) for 72 h significantly ($P$<0.01) caused a 5 to 6 fold increase in the percentage of MHC II positive cells compared with unstimulated SMCs. Treatment with the Eα peptide (100 μg/mL) induced an increase in the percentage of GFP positive SMCs, which was significant at 48 h ($P$<0.05), both in presence or absence of IFN-γ, being indicative of antigen uptake. No significant changes were observed in the percentage of Y-Ae positive SMCs after IFN-γ-stimulation and/or treatment with the Eα peptide, suggesting that, although SMCs are able to internalize protein antigens, they fail to present the Eα peptide in the context of MHC II.
Importantly, neither SMC stimulation with IFN-γ nor treatment with ovalbumin (OVA) antigen or $OVA_{323-339}$ peptide affected proliferation of co-cultured OVA-specific Tg T cells, demonstrating that SMCs fail to support Ag-specific T cell activation.
Finally, we examined whether SMCs express costimulatory molecules. Unstimulated SMCs expressed CD54 (ICAM-1), CD80 and CD44 (30%, 11% and 87% positive cells, respectively). IFN-γ stimulation for 72h significantly caused a 2 fold increase in the percentage of both ICAM-1 and CD80 positive cells ($P$<0.01 and $P$<0.001, respectively) while it had no effect on the expression of CD44. In contrast, low levels of OX40L, CD40, CD70 and CD86 were detectable in both control and stimulated SMCs.

Our results demonstrate that murine primary aortic SMCs are not able to present protein antigens in the context of MHC II. Furthermore, they lack essential costimulatory molecules such as OX40L, CD40, CD70 and CD86.

Itano et al. (2003). *Immunity*. 19, 47-57.
Lötzer et al. (2010). *Arterioscler Thromb Vasc Biol.* 30:395-402.
Hansson et al. (1986). *Clin Exp Immunol.* 64:261–268.