cells (transplantation of Apoe−/− aortas into CCL5−/− Apoe−/− mice).

Conclusion: These results indicate that CCL5 expressed in cells of the vascular wall plays an important role in the recruitment of monocytes / macrophages to transplanted vessels and may thereby contribute to the development of atherosclerotic lesions after aortic transplantation.

Endogenous Toll-like Receptor 4 Deletion Significantly Improves Wound Healing in a Murine Model of Diabetic-ischaemic Ulceration

M.J. Portou

University College London, UK

Introduction: Diabetic foot ulceration is a multifactorial, common and challenging complication of diabetes. Between 15% and 25% of diabetics develop ulceration in their lifetime, leading to a 20 times greater risk of major amputation. There is increasing recognition that toll-like receptor 4 (TLR4) mediated inflammation is involved in the systemic pathogenesis of diabetes and may contribute to impairment of wound healing. We have previously shown in vitro that hyperglycaemia and ischaemia act synergistically to impair fibroblast function and survival via the pro-inflammatory MyD88-dependent pro-inflammatory TLR4 pathway. Here, we aim to study the effect of TLR4 knock-out (KO) on wound healing in a murine diabetic-ischaemic model of hindlimb ulceration.

Methods: Diabetes was induced in wild-type (WT) and TLR4 KO C57BL/6 male mice by intra-peritoneal injection of low-dose streptozocin (STZ 50mg/Kg/day for 5 days) and was confirmed at day 14 by tail capillary blood sampling (BM of >16mmol/l). Glycosuria was monitored with weekly urinalysis. Hindlimb ischaemia was induced by femoral artery ligation at four weeks post STZ, and a full thickness 4mm skin wound inflicted below the knee. Wound healing was assessed via digital planimetry at days 3, 7 and 14 post surgery.

Results: Diabetic-ischaemic wounds demonstrated significantly impaired wound healing compared to diabetic non-ischaemic, ischaemic non-diabetic or non-diabetic, non-ischaemic wounds at day 14 ($p < 0.05$). There was no significant difference in healing rate between diabetes only and ischaemia only wounds. Diabetic-ischaemic wounds in TLR4 KO mice demonstrated significantly improved healing rates compared to those in WT mice at days 7 and 14. 67% of wounds were completely healed in TLR4 KO mice compared to 0% in WT mice by day 14.

Conclusion: Our in vivo model demonstrated significantly impaired wound healing in diabetic-ischaemic animals. In WT animals, no hind-limb wound had completely healed at day 14, suggesting this is a successful model of chronic diabetic ulceration. In TLR4 KO mice, a significant and dramatic improvement in the healing of diabetic-ischaemic wounds was observed.

We conclude that endogenous TLR4 deletion confers a beneficial effect on wound healing in diabetic-ischaemic conditions in vivo, and suggests a potential therapeutic role for TLR4 antagonism in chronic diabetic ulceration.

Pericytes and OPG/RANK/RANKL Triad Contribute to Osteoid Metaplasia Formation in Femoral Arteries

T. Quillard

INSERM UMR957, France

Introduction: Arterial calcification, a process recapitulating bone formation, represents an independent risk of cardiovascular morbidity and mortality and dramatically impacts open and endovascular procedures and outcomes. Research efforts have mainly focus on the coronary and carotid arteries, which are paradoxically less predispose to calcification than other arterial beds. Clinical and mechanistic data regarding calcification structures in femoral arterial territory remain scarce.

Methods: Femoral and carotid endarterectomy samples as well as clinical data, and plasma of a cohort of patients were harvested prospectively. Histological analysis characterized the extent and nature of calcifications present in the lesions. ELISA and cell culture assays were conducted to understand the cellular and the molecular mechanisms underlying the formation of bone-like tissue in the lesions.

Results: Twenty-eight of the 43 femoral plaques (65%) displayed bone-like arterial calcification, named osteoid metaplasia (OM), characterized by osteoid matrix, and osteocytes surrounding bone marrow-like formations. OM was much less frequent in carotid arteries (14%). As in bone, osteoprotegerin (OPG) − a key inhibitor of osteoclast differentiation − was significantly associated with the presence of OM ($p = 0.036$). Likewise, a high plasmatic OPG/ Receptor Activator of NFκ-B Ligand (RANKL) ratio significantly associated with the presence of OM ($p = 0.027$). The histological analysis showed an enriched presence of pericytes in OM+ lesions. In vitro, human primary pericytes secrete considerable amounts of OPG and can actively participate to calcification through their differentiation into an osteoblastic phenotype. In addition to this direct effect, pericytes can also indirectly regulate tissue mineralization via an OPG-dependent inhibition of CD14+ cell osteoclastic differentiation.

Conclusion: Our results show that bone-like arterial calcification (OM) is highly prevalent at the femoral level. At the cellular level, pericytes are associated with the presence of OM. Pericytes are able to undergo an osteogenic differentiation, and indirectly regulate activity of osteoblastic and osteoclastic precursors by secreting OPG.

Altogether, this study demonstrates the active implication of pericytes and OPG/RANKL/RANK triad in bone-like arterial calcification in femoral arteries.

Arterial and Venous Invasion after Intraluminal Injection of Oral Bacteria (P. gingivalis) in a Rat Model shows Buerger Disease Pathology

T. Iwai, H. Terasaki, A. Aoyama, Y. Izumi, M. Umeda, S. Heima, Y. Inoue, M. Fujiwara

Tsukuba Vascular Center, NPO Buerger Disease Research Institute, Japan

Introduction: Porophyromonas gingivalis (Pg) is one of the most representative oral bacteria found in periodontal