

# PostScript

## CORRESPONDENCE

### Differential expression of CD150 (SLAM) on monocytes and macrophages in chronic inflammatory contexts: abundant in Crohn's disease, but not in multiple sclerosis

Signalling lymphocytic activation molecule (SLAM, CD150), originally identified as a lymphocyte activation molecule, is now known to be expressed also on mature dendritic cells and on activated monocytes. Importantly, SLAM is distinct from other monocyte activation markers because its expression on monocytes is readily induced in vitro by bacteria derived ligands of Toll-like receptors (TLRs), and not by single stimulation with inflammatory cytokines.<sup>1</sup>

This study analysed SLAM expression on monocytes and macrophages in two distinct chronic inflammatory pathologies. In Crohn's disease, the chronic inflammation in the gut

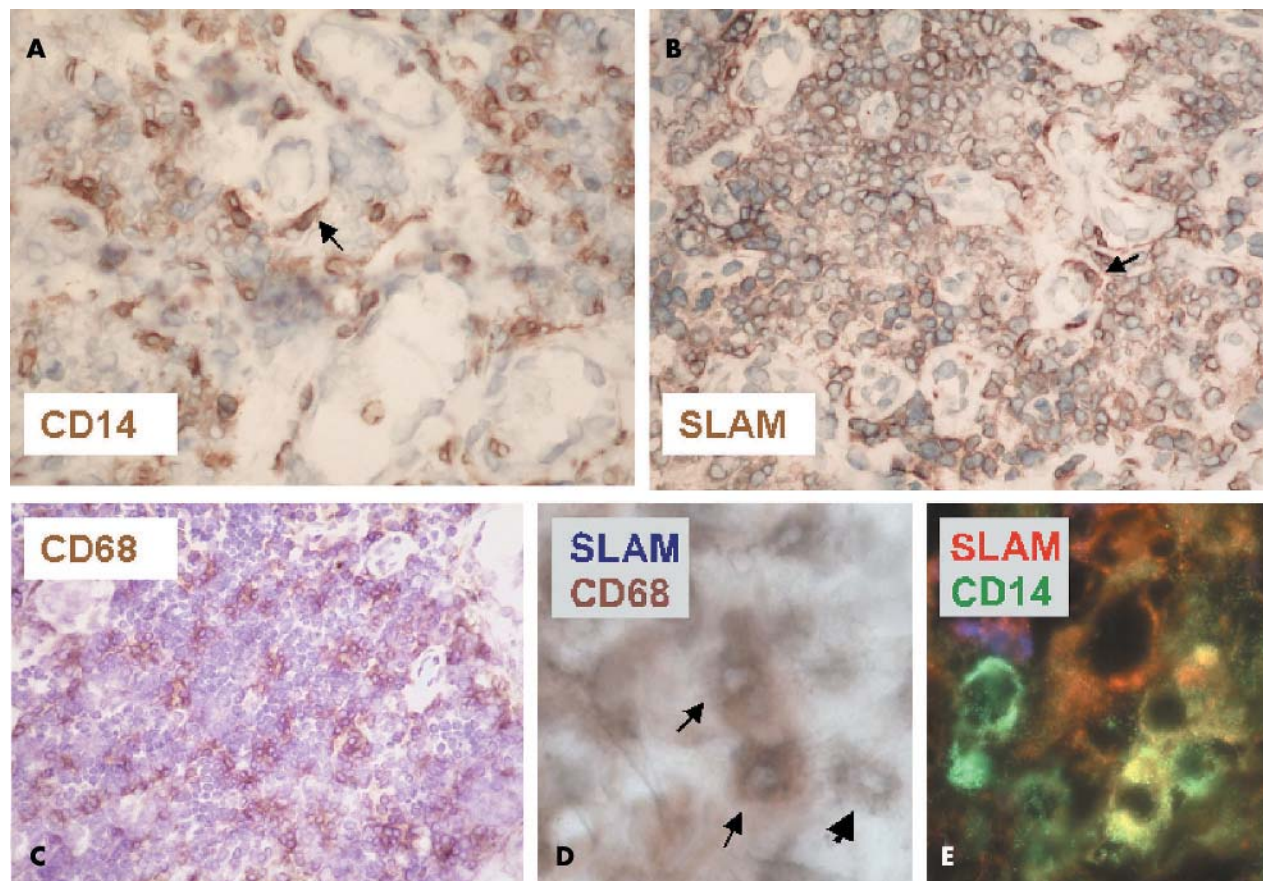
is thought to result from inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora. The bacterial product flagellin has been suggested to be a dominant antigen in Crohn's disease.<sup>2</sup> Flagellin is the ligand for TLR5 and induces SLAM on monocytes.<sup>1</sup> In contrast to Crohn's disease, in multiple sclerosis (MS), the chronic inflammation in the central nervous system is commonly assumed not to be linked to bacterial infections, but rather is driven by autoreactive T cells that recognise self antigens.<sup>3</sup>

Frozen tissue sections from two normal colons, two normal ileums, colon specimens from two patients with Crohn's disease, two lesions from one patient with MS (case 2 in Babbe and colleagues<sup>4</sup>), and three normal brains, were stained with the following monoclonal antibodies: anti-CD14-biotin (BMA Biomedicals, Augst, Switzerland), anti-SLAM (Kamiya Biomedical, Seattle, Washington, USA), and anti-CD68 (Dako, Hamburg, Germany).

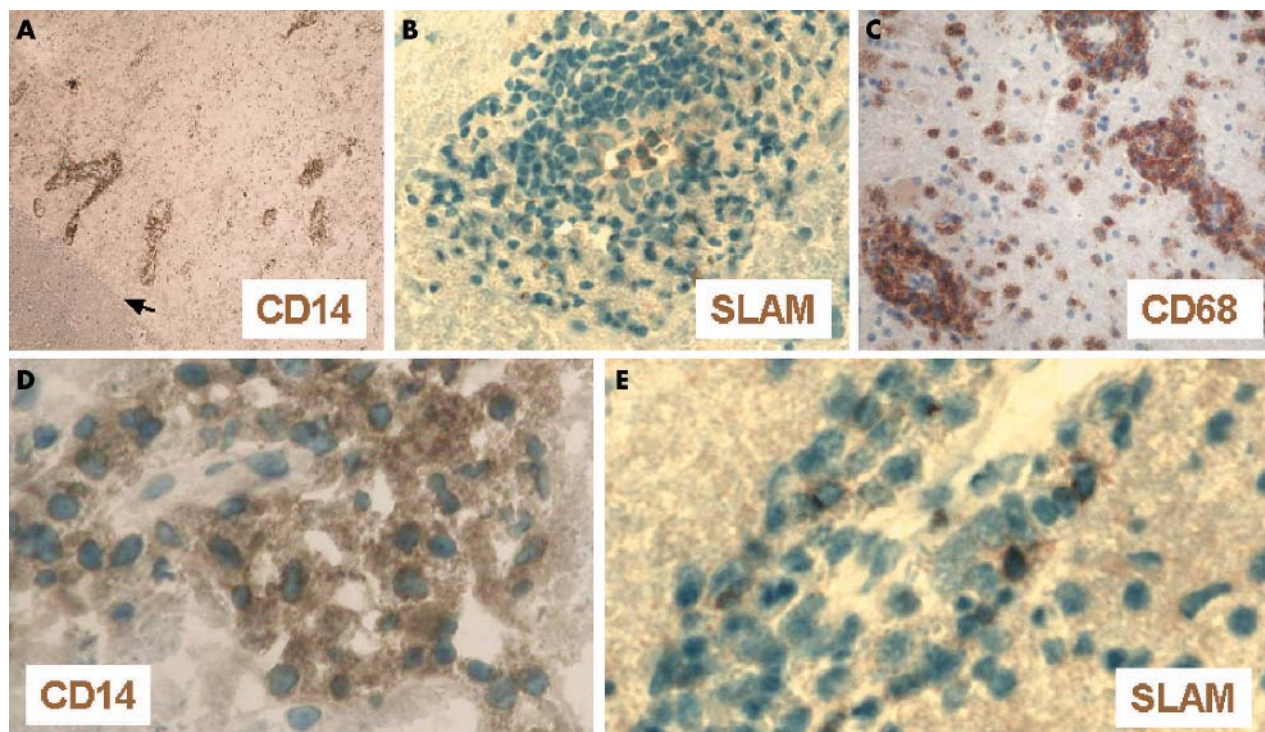
Considerable numbers of CD68+ macrophages and to a lesser extent CD14+ cells

were present in the wall of the normal gut and in Peyer's patches. SLAM+ cells were detected only in Peyer's patches, and not in other parts of the normal tissue (not shown). Numerous CD14+ monocytes, CD68+ macrophages, and SLAM+ cells were detected throughout the inflamed gut of patients with Crohn's disease (fig 1A-C). Some SLAM+ cells were round, but many had processes, indicating that they belonged to the monocyte/macrophage lineage (fig 1B). Double staining demonstrated SLAM expression on CD14+ monocytes and on CD68+ macrophages (fig 1D, E).

In normal brain specimens very few CD14+, CD68+, and SLAM+ cells were detected (not shown). The MS lesion was characterised by an active plaque and composed mainly of activated monocytes and macrophages (fig 2A, C, D). The CD68+ cells extended into the parenchyma and together with CD14+ cells surrounded the inflamed blood vessels, where only very few SLAM positive cells were detected (fig 2B, E). Thus, most, if not all, CD68+ and CD14+ cells did not express SLAM in this MS tissue lesion.



**Figure 1** Expression of CD14, CD68, and SLAM in Crohn's disease. Antibodies to (A) CD14, (B) SLAM, and (C) CD68 showed extensive accumulation of inflammatory cells in the mucosa of the gut. Elongated cells with processes were seen after staining for both CD14 and SLAM (arrows in A and B). Cell nuclei were counterstained with haematoxylin in A-C. (D) Immunohistochemical staining showing blue labelling of SLAM expressing cells and brown labelling of CD68 expressing cells. Two CD68+ cells coexpressing SLAM (arrow) and one cell expressing SLAM only (arrowhead) are labelled. (E) Double staining with anti-CD14 and anti-SLAM revealed cells that were positive for CD14 only (green), positive for SLAM only (red), and double positive (yellow). Original magnification:  $\times 400$  (A-C);  $\times 1000$  (D, E).



**Figure 2** Expression of CD14, CD68, and SLAM in multiple sclerosis (MS). Staining for (A, D) CD14, (B, E) SLAM, and (C) CD68 is shown in an active MS lesion. The sharply demarcated lesion edge is visible (arrow in A). (A, C, E) Numerous CD14+ and CD68+ cells were densely packed around the blood vessels. (C) CD68 expressing cells also infiltrated the parenchyma. (B, E) A few SLAM positive cells can be seen around the blood vessels, but they do not extend into the parenchyma. In all sections nuclei were counterstained with haematoxylin. Original magnification:  $\times 100$  (A);  $\times 400$  (B–E).

Further studies taking into account the pathological heterogeneity of MS<sup>2</sup> are required to elaborate this.

Thus, our study demonstrates for the first time the presence of SLAM+ monocytes and macrophages in a chronic inflammatory disease, Crohn's disease. Furthermore, we show that this is not a feature common to all types of inflammation, because monocytes and macrophages did not express SLAM in the analysed MS lesions. The expression of SLAM on monocytes and macrophages in Crohn's disease supports the concept that the inflammation in this disease is driven by bacteria derived TLR ligands.

### Acknowledgements

Supported by the DFG (SFB 571). The Institute for Clinical Neuroimmunology is supported by the Hermann and Lilly Schilling Foundation. We thank Professor G Niedobitek (University of Erlangen, Germany), Dr M Heiss, Dr H Hartl, and I Paripovic (University of Munich, Germany) for their help, Ms J Benson, Drs A Flügel, and A Holz for helpful comments on the manuscript.

#### D Theil

Department of Neurology, Ludwig-Maximilians University, D-81377 Munich, Germany

#### C Farina

Department of Neuroimmunology, Max-Planck-Institute of Neurobiology, D-82152 Martinsried, Germany; meinl@neuro.mpg.de

#### E Meinel

Institute for Clinical Neuroimmunology, Ludwig-Maximilians University

The first two authors contributed equally to this work.

### References

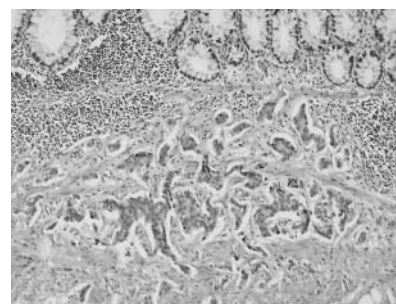
- 1 Farina C, Theil D, Semlinger B, *et al*. Distinct responses of monocytes to Toll-like receptor ligands and inflammatory cytokines. *Int Immunol* 2004;**16**:799–809.
- 2 Lodes MJ, Cong Y, Elson CO, *et al*. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest* 2004;**113**:1296–306.
- 3 Hemmer B, Archelos JJ, Hartung HP. New concepts in the immunopathogenesis of multiple sclerosis. *Nat Rev Neurosci* 2002;**3**:291–301.
- 4 Babbe H, Roers A, Waisman A, *et al*. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 2000;**192**:393–404.
- 5 Lucchinetti C, Brück W, Parisi J, *et al*. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000;**47**:707–17.

### Multifocal microcarcinoid tumours in ulcerative colitis

I read with interest the report by Matsumoto and colleagues<sup>1</sup> of a patient with ulcerative colitis and multiple microcarcinoid tumours, and would like to describe a similar recent case encountered in our department. A 44 year old man with an 18 year history of ulcerative colitis underwent total colectomy after a diagnosis of high grade dysplasia in a rectal biopsy. The resection specimen revealed a mucinous adenocarcinoma of the rectum invading the submucosa, but not involving the muscularis propria, perirectal fat, or lymph nodes. There was also active ulcerative colitis of mild to moderate severity involving the distal 200 mm of the colon and rectum. The inflamed segment was extensively sampled and all sections showed

multifocal endocrine cell proliferation within the muscularis mucosae and the superficial submucosa (fig 1). Most foci comprised clusters of between five and 50 cells, which were typically arranged in small nests and cords, but there were four larger endocrine cell aggregates from 1 to 2.5 mm maximum diameter. Some of the submucosal nests were closely apposed to nerves and ganglion cells. Immunohistochemistry on representative sections showed expression of cytokeratin, chromogranin, synaptophysin, and CD56 within the endocrine cells and close apposition to S100 protein positive nerves and dendritic cells. There was no evidence of endocrine cell hyperplasia within the overlying mucosa or in the normal proximal bowel.

Although there are several reports of neuroendocrine tumours arising in patients with inflammatory bowel disease, the finding



**Figure 1** Endocrine cell proliferation within thickened rectal muscularis mucosae. There is no evidence of mucosal endocrine hyperplasia.

of microscopic and multifocal endocrine cell proliferations is much more rare. Indeed, the most appropriate terminology for these lesions is not clear. Matsumoto and colleagues<sup>1</sup> used the term "microcarcinoids" to describe the endocrine proliferations in their patient. The lesions were described as "multiple small islands of cells" but the sizes of the lesions were not documented. Maruyama and colleagues<sup>2</sup> described a non-colitic patient with multiple, variably sized endocrine cell proliferations and five typical grossly evident carcinoid tumours in the colon. They suggested that the microscopic lesions should be subdivided into microcarcinoids, endocrine cell microproliferations (ECMPs), and transitional forms. The smaller ECMPs and transitional lesions did not exhibit expansile growth or intervening fibrous stroma. Abe *et al.*,<sup>3</sup> in describing a case of multiple duodenal endocrine cell proliferations, defined carcinoid tumours as greater than 3 mm diameter, microcarcinoids as between 0.5 and 2.9 mm, and lesions smaller than 0.5 mm (which they interpreted as hyperplastic) as "endocrine cell micronests". According to these criteria, most of the lesions in our case would be classified as ECMPs or micronests. Regardless of the terminology, it would appear that these cases show a spectrum of endocrine cell proliferation, but whether these are truly hyperplastic or neoplastic is not clear.

As discussed by Matsumoto *et al.*,<sup>1</sup> it has been suggested previously that colonic endocrine cell hyperplasia in association with inflammatory bowel disease may represent a response to injury or inflammation. However, although this hypothesis may reasonably explain mucosal endocrine cell proliferation, the distribution of the lesions within the submucosa and muscularis mucosae in our patient and in some previous reports<sup>1,2,4</sup> is less readily understood, particularly because the crypt endocrine population was not hyperplastic in these cases. Maruyama and colleagues<sup>2</sup> noted in their case that the submucosal endocrine cells were often associated with ganglion cells and S100 positive dendritic cells, and postulated that the proliferation involved a subpopulation of endocrine cells associated with submucosal nerve fibres. This suggestion is supported by the findings in our case. As noted by Matsumoto *et al.*,<sup>1</sup> deeply located endocrine cell proliferations are unlikely to be identified on endoscopic biopsies. Indeed, our patient underwent four colonoscopic examinations in the 12 months before surgery and no endocrine lesions were identified in a total of 24 biopsy samples examined during that period. Therefore, the incidence of such lesions may be underestimated, although whether this is a clinically relevant finding is not clear.

#### C J R Stewart

Department of Histopathology, King Edward Memorial Hospital, Bagot Road, Subiaco, Perth, Western Australia 6009

#### References

- 1 Matsumoto T, Jo Y, Hirahashi M, *et al.* Multiple microcarcinoids in a patient with long standing ulcerative colitis. *J Clin Pathol* 2003;**56**:963-5.

- 2 Maruyama M, Fukayama M, Koike M. A case of carcinoid tumors of the rectum with extraglandular endocrine cell proliferation. *Cancer* 1988;**61**:131-6.
- 3 Abe H, Kubota K, Oka T, *et al.* A rare case of multiple carcinoids and endocrine micronests in a patient with chronic duodenitis. *Cancer* 2000;**89**:63-9.
- 4 McNeely B, Owen DA, Pezim M. Multiple microcarcinoids arising in chronic ulcerative colitis. *Am J Clin Pathol* 1992;**98**:112-16.

#### Authors' reply

We appreciate the valuable comments regarding our case report published in the December 2003 issue of the journal.<sup>1</sup> We are impressed by a similar case of ulcerative colitis complicating microcarcinoids described above.

As has been stated above, there are no established criteria that distinguish microcarcinoids from endocrine cell micronests (ECMs) occurring in the intestine. Thus, the terminology seems to be confused. However, the diagnosis of microcarcinoids in our case was based on the criteria for gastric carcinoids reported by Itsuno *et al.*<sup>2</sup> For an analysis of autoimmune gastritis, Itsuno and colleagues<sup>2</sup> defined microcarcinoids as lesions composed of endocrine cells over 0.5 mm, and ECMs as lesions between 0.1 and 0.5 mm in size. Because all the lesions in our case measured over 0.5 mm, and they consisted of crowding micronests, we judged them to be "microcarcinoids". We apologise for the insufficient descriptions provided in our report.

Although such a distinction has not been shown to be appropriate for endocrine cell tumours in the large intestine, the application of the criteria seems to be reasonable, because ECMs and microcarcinoids in both autoimmune gastritis and ulcerative colitis are probably a consequence of chronic inflammation. The clinical relevance of the distinction of microcarcinoids from ECMs for the management of ulcerative colitis needs to be evaluated further.

As has been recommended, we performed immunostaining for S-100 protein in the microcarcinoids of our case. However, the procedure failed to identify positive cells within the lesions. Thus, the association of nerve fibres is unlikely to explain the submucosal nature of the microcarcinoids in our case.

#### T Matsumoto, Y Jo

Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

#### R Mibu

Department of Clinical and Surgical Oncology, Graduate School of Medical Sciences, Kyushu University

#### M Hirahashi, T Yao

Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University

#### M Iida

Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University

#### References

- 1 Matsumoto T, Jo Y, Hirahashi M, *et al.* Multiple carcinoids in a patient with long standing ulcerative colitis. *J Clin Pathol* 2003;**56**:963-5.
- 2 Itsuno M, Watanabe H, Iwafuchi M, *et al.* Multiple carcinoids and endocrine cell micronests in type A gastritis. Their morphology, histogenesis, and natural history. *Cancer* 1989;**63**:881-90.

#### BOOK REVIEW

#### Molecular Biology and Pathology of Paediatric Cancer

Edited by C J Cullinane, S A Burchill, J A Squire, *et al.* Oxford: Oxford University Press, 2003, £99.95 (hardback), pp 332.

The diagnosis of cancer, especially in children, has evolved into a multistep process involving many diagnostic techniques. Molecular techniques are now essential tools in the diagnosis of paediatric cancer. This book will update pathologists and clinicians on the current techniques and their application to pathological diagnosis.

The first introductory chapter gives an excellent overview of childhood cancer including patient management. This is followed by two sections, the first covering laboratory methods and techniques. Also included in the first section is a chapter on "familial and predisposition syndromes", which includes disorders of DNA repair and familial cancer syndromes. The second section consists of 12 chapters, which deal mainly with the molecular pathology of the major paediatric cancers, but also include information on the clinical features, pathology, and prognosis of selected neoplasms. The text is well written and complemented by appropriate tables and figures.

A typographical error appears in tables 1.5 and 9.2. Both tables list 11q13, instead of the correct 11p13, in the translocation for desmoplastic small round cell tumour.

I thoroughly enjoyed reading this book. I think that it is an excellent book in its category and would recommend it to scientists, clinicians, and pathologists who wish to understand the recent developments in paediatric cancer diagnosis.

D Govender

#### CALENDAR OF EVENTS

#### Diagnostic Histopathology of Breast Disease

9-13 May 2005, Hammersmith Hospital and Imperial College, London, UK

Further details: Wolfson Conference Centre, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK. (Tel +44 (0)20 8383 3117/3227/3245; Fax +44 (0)20 8383 2428; e-mail wcc@ic.ac.uk)