(LAG-3)-LAP, a lymphocyte activation gene-3 (LAG-3)-associated protein that binds to a repeated EP motif in the intracellular region of LAG-3, may participate in the down-regulation of the CD3/TCR activation pathway. *Eur. J. Immunol.* 31, 2885–2891
14 Huard, B. *et al.* (1995) CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. *Eur. J. Immunol.* 25, 2718–2721
15 Barten, R. *et al.* (2001) Divergent and convergent evolution of NK-cell receptors. *Trends Immunol.* 22, 52–57
16 Huard, B. *et al.* (1998) LAG-3 does not define a specific mode of natural killing in human. *Immunol. Lett.* 61, 109–112
17 Hung, L.Y. *et al.* (2000) Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the γ-tubulin complex. *Mol. Cell. Biol.* 20, 7813–7825
18 Buisson, S. and Triebel, F. (2003) MHC class II engagement by its ligand LAG-3 (CD223) leads to a distinct pattern of chemokine receptor expression by human dendritic cells. *Vaccine* 21, 862–868
19 Prigent, P. *et al.* (1999) LAG-3 induces tumor regression and antitumor immune responses *in vivo*. *Eur. J. Immunol.* 29, 3867–3876
20 Cappello, P. *et al.* (2003) LAG-3 enables DNA vaccination to persistently prevent mammary carcinogenesis in HER-2/neu transgenic BALB/c mice. *Cancer Res.* 63, 2518–2525
21 Setterblad, N. *et al.* (2003) Composition of MHC class II-enriched lipid microdomains is modified during maturation of primary dendritic cells. *J. Leukoc. Biol.* 74, 40–48
22 Machy, P. *et al.* (2002) Induction of MHC class I presentation of exogenous antigen by dendritic cells is controlled by $CD4^+$ T cells engaging class II molecules in cholesterol-rich domains. *J. Immunol.* 168, 1172–1180
23 Buatois, N. *et al.*, MHC class II–peptide complexes in dendritic cell lipid microdomains initiate the CD4 Th1 phenotype. *J. Immunol.* (in press)
24 Lienhardt, C. *et al.* (2002) Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity *in vivo*. *Eur. J. Immunol.* 32, 1605–1613
25 Phan, G.Q. *et al.* (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8372–8377

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Letters

Absence of *S100A12* in mouse: implications for RAGE–S100A12 interaction

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RAGE (receptor of advanced glycation endproducts), a member of the IgG superfamily, is present on numerous cell types, including monocytes, fibroblasts, endothelial and smooth muscle cells. Activation of the RAGE pathway is important in wound healing, atherogenesis, tumorgenesis, systemic amyloidosis and Alzheimer's disease [1,2]. Ligation of RAGE activates multiple intracellular signalling pathways and several downstream signalling molecules have been identified [1]. RAGE also binds the non-AGEs (non-advanced glycation endproducts) S100B and S100A12, termed extracellular newly identified RAGE-binding proteins (EN-RAGEs) [3], and the RAGE–S100 interaction represents a novel proinflammatory axis involved in several inflammatory diseases [3].

Members of the S100–calgranulins family compose a multigenic family of non-ubiquitous cytoplasmic Ca^{2+}-binding proteins of the EF-hand type, differentially expressed in a wide variety of cell types [4,5]. Some members, especially S100A8, S100A9 and S100A12 [6,7], are released from cells as a result of cellular activation. Secreted human S100A12 binds to RAGE on immune cells, promotes proliferation, induces the activation of NF-κB and the expression of several cytokines, such as interleukin-1α (IL-1α), IL-2 and tumor necrosis factor-α (TNF-α). However, although blockage of RAGE–S100A12 suppresses the chronic cellular activation and tissue injury in various mouse models [3,8], there is one important gap: whether murine S100A12 exists is not yet known. Therefore, we performed a detailed computer analysis to identify the murine *S100A12* gene.

The genes encoding 14 of the 21 members of the S100–calgranulins family are localised in a cluster on human chromosome 1q21 and several murine S100 homologues have been localised to a corresponding region on mouse chromosome 3 [9,10]. *S100A8* and *S100A9* are neighbouring genes in both mouse and human, and human *S100A12* is localised in the S100 gene cluster between the *S100A8* and *S100A9* genes [10]. Like most other S100 genes, human *S100A12* is composed of three exons and the protein is encoded by sequences in exons 2 and 3.

The University of California at Santa Cruz (UCSC) genome browser* [11] enabled us to compare the Feb. 2003 freeze of the murine genome with the Nov. 2002 assembly of the human genome. To search for the murine *S100A12* gene, we inspected the murine sequence segment between *S100A8* and *S100A9*. The genome browser displayed seventeen Blastz [12] best genome alignment hits to human. Blastz is an implementation of the Gapped Blast algorithm specifically designed for aligning two long genomic sequences. The hits reveal regions of high sequence similarity between the murine and the corresponding human segment that is also enclosed by *S100A8*

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Figure 1. Hit 9 corresponds to the alignment shown, between the human sequence ranging from 149131088 to 149130541 base pairs on chromosome 1 (top) and murine sequence ranging from 91810347 to 91810828 base pairs on chromosome 3 (bottom). The alignment consists of 315 (60.9%) identical bases. Exon 1 of *S100A12* is marked in red, and the putative TATA box is marked in green.

and *S100A9*. The hits have the same order in both species and cover most of the murine segment. The murine sequence that triggers hit 9 is 60% identical to a region of the human *S100A12* gene covering promoter, exon 1 and intron 1 (Figure 1). The corresponding regions of the *S100A8* and *S100A9* genes share a comparable degree of similarity between mouse and human arguing that hit 9 represents part of murine *S100A12*. However, analysing the murine sequence immediately following hit 9 we failed to detect exons 2 and 3. Instead, we found a region homologous to the human sequence near to the *S100A9* locus, suggesting that the murine *S100A12* exons 2 and 3 have been deleted. Furthermore, there is no evidence for a translocation in mouse because a search with the human sequence following intron 1 of the *S100A12* gene did not trigger any homologous matches. There are no nearby sequencing gaps. Additional homology searches of all Jackson laboratory murine databases [13], using human *S100A12* as the query, were futile. Moreover, a region homologous to the first exon of *S100A12* is also present on the corresponding chromosome 2 of rat, however, exons 2 and 3 are again missing, suggesting that the *S100A12* gene might be damaged in all rodents. However, the high conservation of the *S100A12* relic in mouse indicates a quite recent loss of the *S100A12* gene during rodent evolution. The potentially deleterious A → G substitution in the putative TATA box of the murine *S100A12* promoter (Figure 1) also points to an inactive evolutionary relic of a formerly active gene.

RAGE ligation by its various ligands has been implicated in various inflammatory-related diseases [1,2]. The hallmark of S100A12 is its accumulation at sites of acute and chronic inflammation and human S100A12 is undisputedly associated with inflammation [14]. Therefore, several proinflammatory properties of this protein might be caused by its binding to RAGE [3]. Thus, the RAGE–S100A12 interaction represents an attractive model to explain how RAGE and its proinflammatory ligand contribute to the pathophysiology of several inflammatory diseases [1,3]. Consequently, inhibition of the RAGE–S100A12 interaction could be considered as an attractive target for future therapies.

Blockage of RAGE–S100A12 suppresses chronic cellular activation and tissue injury in various mouse models [3,8]. However, evidence that a functional *S100A12* gene is not present in the murine genome implies that RAGE–S100A12 ligation does not attribute to the molecular mechanisms by which the beneficial effects of blocking the RAGE–ligand interaction in mice are mediated. Although members of the S100–calgranulin family have common

structural features and display sequence homology, there are obvious differences in biochemistry, distribution and expression. Definitive evidence for binding to RAGE has only been deduced for S100A12 and S100B [3]. Therefore, further investigations are required to analyse the molecular mechanisms by which soluble RAGE and anti-RAGE have beneficial effects on inflammation.

References

1 Stern, D. *et al.* (2002) Receptor for advanced glycation endproducts: a multiligand receptor magnifying cell stress in diverse pathologic settings. *Adv. Drug Deliv. Rev.* 54, 1615–1625
2 Deane, R. *et al.* (2003) RAGE mediates amyloid-β peptide transport across the blood–brain barrier and accumulation in brain. *Nat. Med.* 9, 907–913
3 Hofmann, M.A. *et al.* (1999) RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 97, 889–901
4 Kerkhoff, C. *et al.* (1998) Novel insights into structure and functions of S100A8 and S100A9. *Biochim. Biophys. Acta* 1448, 200–211
5 Donato, R. (2003) Intracellular and extracellular roles of S100 proteins. *Microsc. Res. Tech.* 60, 540–551
6 Rammes, A. *et al.* (1997) Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. *J. Biol. Chem.* 272, 9496–9502
7 Boussac, M. and Garin, J. (2000) Calcium-dependent secretion in human neutrophils: a proteomic approach. *Electrophoresis* 21, 665–672
8 Schmidt, A.M. *et al.* (2001) The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J. Clin. Invest.* 108, 949–955
9 Ridinger, K. *et al.* (1998) Clustered organization of S100 genes in human and mouse. *Biochim. Biophys. Acta* 1448, 254–263
10 Wicki, R. *et al.* (1996) Characterization of the human *S100A12* (calgranulin C, p6, CAAF1, CGRP) gene, a new member of the S100 gene cluster on chromosome 1q21. *Cell Calcium* 20, 459–464
11 Kent, W.J. *et al.* (2002) The human genome browser at UCSC. *Genome Res.* 12, 996–1006
12 Schwartz, S. *et al.* (2003) Human–mouse alignments with BLASTZ. *Genome Res.* 13, 103–107
13 Blake, J.A. *et al.* (2003) MGD: the mouse genome database. *Nucleic Acids Res.* 31, 193–195
14 Foell, D. *et al.* (2003) Neutrophil derived human S100A12 (EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease. *Gut.* 52, 847–853

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TCR triggering: co-receptor-dependent or -independent?

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We wish to defend the kinetic-segregation (K-S) model of T-cell receptor (TCR) triggering [1,2] against several points raised by Trautmann and Randriamampita in their recent Opinion article on the initiation of TCR signalling [3]. The authors claim that the K-S model is weakened by apparently failing to account for co-receptor dependent signalling, is experimentally unsubstantiated and cannot explain the signalling effects of soluble MHC–peptide molecules.

It seems worth explaining why the K-S model was intentionally formulated without an obligatory role for co-receptors. At the time it was first proposed [1], the somewhat surprising view had emerged that TCR signalling was not invariably co-receptor dependent, as first vividly illustrated by the control of leishmaniasis by $CD8^-$ Th cells in CD4-deficient mice [4]. Molecular analyses also indicated that TCR signals enhance the binding of CD8 to MHC class I [5] and of CD4 to the TCR [6]. Unexpectedly, the co-receptor function of CD4 was potentiated by removal of the kinase domain of CD4-associated $p56^{lck}$ and eliminated by inactivation of the Src homology 2 (SH2) domain from a kinase-inactive mutant [7]. Together, these observations suggested that, instead of TCR phosphorylation requiring CD4 or CD8 recruitment, co-receptor recruitment depended on prior phosphorylation of the TCR. Today, the observation that TCRs are triggered even when co-receptors are blocked hardly seems to warrant comment (see e.g. Ref. [8]).

The K-S model avoided an absolute requirement for co-receptors by allowing TCR phosphorylation (triggering) by free $p56^{lck}$ associated with the membrane inner leaflet (Figure 1). This does not mean that signalling is necessarily co-receptor independent, however. We initially envisaged that the co-receptors and other non-crucial accessory molecules, such as CD2, might enhance signalling by stabilizing TCR–MHC–peptide complexes [1]. However, the extremely low affinities of co-receptor interactions make this unlikely [9]. Instead, co-receptors might stably recruit $p56^{lck}$ to incipiently phosphorylated TCRs through additional SH2 domain-dependent interactions, allowing the amplification of signalling beyond a critical threshold [9,10]. This might ensure that potent, cell-mobilizing co-receptor signalling is subservient to weaker, but fundamentally more important, TCR-derived signals.

Trautman and Randriamampita claim that there is little experimental support for the K-S model but this is not the case. Along with its compatibility with several long-standing features of TCR triggering [2],

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