

## Necrosis-Associated Factors (DAMPs) Including S100A4 Used to Pulse Dendritic Cells (DCs) Induce Regulatory T cells

Wiegmann, Lisa Asseck, Alexander Erle, Tatjana Yildiz, Bernd Jahrsdörfer and Hubert Schrezenmeier

Head of the Department for Innovative Cellular Therapeutics, Institute of Transfusion Medicine, University Hospital Ulm, Helmholtzstraße 10, 89081 Ulm, Germany

### Abstract

Emerging data support the immunosuppressive nature of the tumour microenvironment, which interferes with the efficacy of Dendritic Cells (DCs) used in cancer vaccination not only by rendering these immune cells unable to induce specific immune responses, but also by turning them into promoters of tumour growth.

A characteristic feature within the tumour microenvironment of advanced solid tumours is the necrotic cell death with subsequent release of necrosis-/Damage-Associated Molecular Patterns (DAMPs). DAMPs are known to induce tissue healing processes including angiogenesis and immune suppression, thus necrotic tumours harness the regenerative capacities of the host for their own survival and proliferation.

We examined the impact of DAMPs including S100A4 on DC-induced T cell proliferation. Human monocyte derived DCs were pulsed with DAMPs obtained from necrotic tumour material or the specific DAMP-member S100A4. Pulsed DCs were subsequently co-cultured with autologous T cells. We could show enhanced metabolic activity and proliferation of CD4+CD25+FoxP3+regulatory T cells, which were capable of suppressing allogeneic lymphocytes in the mixed lymphocyte culture.

Given that necrotic material is generally found within advanced tumour tissue, there is an urgent need to characterize specific members of DAMPs and their impact within the tumour microenvironment. By pointing out the crucial effect of S100A4 protein our results shed some light into the underlying mechanisms playing important roles in adaptive immune response to tumours. Given that S100 proteins are sensitive to oxidation it is conceivable that induction of oxidative microenvironmental conditions within the tumour tissue could abrogate necrosis induced immune suppression.

**Keywords:** Necrosis; Damage associated molecular pattern; DAMPs; Dendritic cells; Regulatory T-cells

### Introduction

Considerable efforts have been made in order to develop strategies for using DCs to induce tumour-specific immunity, including more than 100 clinical trials designed to evaluate their safety or efficacy in humans [1]. In general, although several reports indicate that DC vaccines are able to induce immune responses in cancer patients, they only rarely result in objective clinical responses based on the response evaluation criteria in solid tumours (RECISTs) and no indication or evidence has been obtained supporting the notion that DC vaccines are superior to other vaccination strategies for cancer patients [2]. One of the main reasons why DC vaccines have been suboptimal in clinical trials might be the inhibitory effect of the tumour microenvironment. Indeed, tumour-associated DCs are usually described as immature cells with low expression of co-stimulatory molecules and incapable of inducing robust anti-tumour immune responses [3-7].

Feijoo et al. [8] showed in a clinical trial that ex vivo matured DCs, loaded with tumour antigen, are trapped by the tumour microenvironment and thus incapable of migrating into regional lymph nodes. Tumour-associated DCs are often incapable of inducing specific cytotoxic immune responses, or can even induce regulatory T cell expansion. In particular, DCs showing low levels of co-stimulatory molecules have been detected in tumours expressing high levels of VEGF [9] resulting in enhanced angiogenesis with the improved nutrient supply to the tumour that supports tumour growth and proliferation.

Current strategies to circumvent the immunosuppressive influence of the tumour microenvironment target either local bioactive factors

as cytokines and chemokines, or tumour-associated cells like myeloid derived suppressor cells and regulatory T cells (T regs). Indeed, depletion of Tregs before vaccination with Carcino Embryonic Antigen (CEA) pulsed DCs can enhance specific T-cell response to CEA [10], similar effect of Treg depletion could be observed in patients with metastatic renal cell carcinomas [11], underlining the deleterious effect of tumour associated Tregs for the host.

Besides cytokines and chemokines which are released from tumour cells or tumour-associated cells, Damage-Associated Molecular Patterns (DAMPs) belong to the prevailing bioactive factors which critically impact the tumour microenvironment by enhancing angiogenesis or influencing the immune response [12,13]. Necrotic cell death with subsequent release of DAMPs is a characteristic feature of advanced solid tumours regardless of the origin and site of neoplastic cells. Necrosis (thus the release of DAMPs) is found within the tumour tissue due to three circumstances: 1) inadequate nutrient supply to tumour cells as a consequence of an imbalance between tumour growth and angiogenesis, 2) the host's cytotoxic immune response to the

**\*Corresponding author:** Ramin Lotfi, Assistant Professor, Head of the Department for Innovative Cellular Therapeutics, Institute of Transfusion Medicine, University Hospital Ulm, Helmholtzstraße 10, 89081 Ulm, Germany, Tel: +49-731-150-6851; Fax: +49-731-150-6574; E-mail: [r.lotfi@blutspende.de](mailto:r.lotfi@blutspende.de)

**Received** November 19, 2012; **Accepted** December 17, 2012; **Published** December 19, 2012

**Citation:** Lotfi R, Wiegmann DS, Asseck L, Erle A, Yildiz T, et al. (2012) Necrosis-Associated Factors (DAMPs) Including S100A4 Used to Pulse Dendritic Cells (DCs) Induce Regulatory T cells. J Cell Sci Ther 3:134. doi:10.4172/2157-7013.1000134

**Copyright:** © 2012 Lotfi R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

tumour, and 3) downregulation of programmed (apoptotic) cell death by the tumour itself [13]. Identified DAMPs –shared by almost all cell types - include high mobility group box 1 (HMGB1), uric acid, ATP, hyaluronan, heat shock proteins, heparan, syndecan, versican, and S100 proteins [14].

S100A4 is a member of the S100-calcium-binding family, and is involved in regulation of angiogenesis, cell motility, invasion and cell survival [15]. The expression of S100A4 is known to be increased in colorectal cancer cells [16], and its elevated concentration within the tumour tissue or in serum is indicative of tumour progression and lymph node metastasis with subsequent poor prognosis for the patients [15-18].

Given that S100 proteins are sensitive to oxidation, and considering our published observations that oxidized DAMPs lose their biologic activity [19,20], we focussed our further experiments on S100A4 as a DAMP member being possibly responsible for observed effects.

In our previous studies we could show that DAMPs obtained from lysed colorectal tumour cells are capable of inducing the maturation of human monocyte derived DCs in terms of up-regulation of CD40, CD80, and CD86 [21]. We could also demonstrate that DAMPs are capable of enhancing the chemotaxis and proliferation of mesenchymal stromal cells (MSCs) [20]. MSCs accumulate within solid tumours and are associated with a poor prognosis of cancer patients possibly by virtue of their immunosuppressive capacities [22]. We now sought to determine the impact of S100A4 on the biologic activity of DCs encountering necrosis within the tumour tissue assuming that S100A4 may be responsible for the induction of regulatory DCs promoting generation of Tregs within the tumour.

## Materials and Methods

### Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Institutional Review Board (Ethical Committee) of the University of Ulm.

### Standard cell culture conditions and used cells

As standard cell culturing media phenol-red free DMEM (Gibco) supplemented with 10% human serum of blood group AB (German Red Cross Blood Services Baden-Württemberg-Hessen) was used. All cells (HCT-116, CACO-2, monocytes, dendritic cells, lymphocytes) were incubated in a humidified atmosphere at 37°C with 5% CO<sub>2</sub>.

The colorectal tumour cell lines HCT-116 and CACO-2 which were lysed to obtain necrotic material were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig).

### Quantitative real time RT-PCR for detection of *Homo sapiens* S100A4 (Entrez Gene ID: 6275) transcripts

For quantitative detection of S100A4 the following TaqMan® Gene Expression Assays from Applied Biosystems® (Foster City, U.S.A) were used: S100A4=Hs00243202\_m1, GAPDH = Hs02758991\_g1. For total RNA extraction QIAGEN® RNeasy® Mini Kit (Cat No. 74104) in combination with QIAGEN® RNase-Free DNase Set (Cat No. 79254) and for reverse transcription reaction QIAGEN® Omniscript® RT Kit (Cat No. 205111) was used. Quantitative PCR was performed on an Applied Biosystems® 7500 Fast Real-Time PCR System by using Applied Biosystems® TaqMan® Fast Universal PCR Master Mix (2X),

No AmpErase® UNG (Part No. 4352042). All kits were used according to the manufacturers' manuals. Data were analysed using 7500 System SDS v2.0.4 and Microsoft Office Excel 2003 software.

### Preparation of cell lysates and soluble DAMPs

Colorectal tumour cell lines HCT-116 and CACO-2 were cultured in our standard cell culturing media containing human (not bovine) serum (see above). At confluencies between 70% and 80%, adherent cells were harvested using trypsin/EDTA (0.05/0.02%). Remaining trypsin, culturing media and possibly contaminating necrotic material from dead cells was removed by washing and resuspending the harvested viable cells in sterile PBS (Lonza). At concentrations of 10<sup>7</sup> to 10<sup>8</sup> HCT-116 cells/ml PBS, cells were lysed by 4 cycles of freeze-thawing (F/T). The viability following F/T treatment was assessed using trypan blue exclusion and was always below 0.1%. In order to obtain soluble DAMPs, lysates were spun down hard (16,300 × g) twice and the supernatants (soluble DAMPs) were used at indicated concentrations, alternatively recombinant human S100A4 (R and D systems) was used to pulse Dendritic Cells (DCs).

Total protein concentrations within generated soluble DAMPs was assessed by using a BCA protein assay kit (Thermo Scientific Pierce Protein Biology Products, Germany) according to manufacturer's instructions.

Monoclonal mouse anti human S100A4 (Genovac, Germany) at indicated concentrations was added to the cell culture in order to deplete S100A4 from the necrotic material.

### Isolation of peripheral blood mononuclear cells and purification of monocytes and autologous lymphocytes

Human peripheral blood mononuclear cells (PBMC) were purified from whole blood by density gradient centrifugation using Biocoll (Biochrom AG, Germany). Monocytes (CD14-positive selection) and CD4+ lymphocytes (CD4-negative selection) were purified from PBMC by performing MACS procedure (Miltenyi Biotec, Germany) following manufacturer's instructions.

### Generation of monocyte derived human DCs

Immature DCs (iDCs) were generated from human monocytes within 5 days in the presence of IL-4 (500 U/ml) and GM-CSF (1000 U/µl) [23] (both cytokines from R&D Systems). iDCs were harvested by using EDTA, resuspended in our standard media and transferred into a flat bottom 24 well plate (Nunc) at a density of 3 to 4 × 10<sup>5</sup> iDCs per well and 500 µl. iDCs were stimulated with indicated concentrations of individual tumour cell lysates for 3 further days and harvested then by using EDTA to detach the DCs.

### Flow cytometric assessment of marker expression on lymphocytes

Fluorescent labelled antibodies to human CD4, CD25, FoxP3, respective isotype controls as well as cell fixation and permeabilization buffers were purchased from BD Biosciences. The fluorescence of stained cells was assessed using BD FAC Scan (BD Biosciences). At least 20,000 events were acquired in all flow cytometric analyses.

### Assessment of lymphocyte proliferation and metabolic activity

DCs (10<sup>4</sup> /well) which had previously been stimulated with tumour cell lysate (DAMPs) for 3 days were co-cultured with 10<sup>5</sup> fluorescent-labelled (Cell Trace from Invitrogen) CD4+ autologous lymphocytes

in a 96 well plate. After a week floating/non-adherent lymphocytes were separated from culture flask-adherent DCs by careful pipetting and residual contaminating DCs were gated out based on their side and forward scatter when performing flow cytometry. Cell division correlated with loss of fluorescence signal which was assessed using BD FACScan (BD Biosciences).

In order to assess viability and metabolic activity, WST-1 assay (Sigma Aldrich) was performed according to manufacturer's recommendations. Like other tetrazolium salts, WST-1 is reduced and thus cleaved to formation due to the mitochondrial dihydrogenase activity of cells. An expansion in the number of viable cells results in an increase in the overall enzyme activity leading to an increased amount of formazan dye resulting in enhanced absorbance which is measured at 450 nm. The WST-1 assay was used to confirm lymphocyte proliferation but also to assess metabolic activity which being a measure for performance-is best indicated as WST-1 cleavage/h. Given the non-dividing nature of DCs and considering that the number of contaminating DCs was more than tenfold lower, WST-1 assays were performed without separating DCs from lymphocytes resulting in a small background noise which was corrected by calculating the relative (%) increase of WST-1 cleavage, as indicated in the figures.

Mixed lymphocyte reaction was performed for 7 days by adding  $10^5$  allogeneic HLA-A2 mismatched PBMC to  $10^5$  lymphocytes which were previously co-cultured with DAMP-pulsed autologous DCs as mentioned above. Autologous lymphocytes were distinguished from

allogeneic lymphocytes based on their HLA-A2 expression.

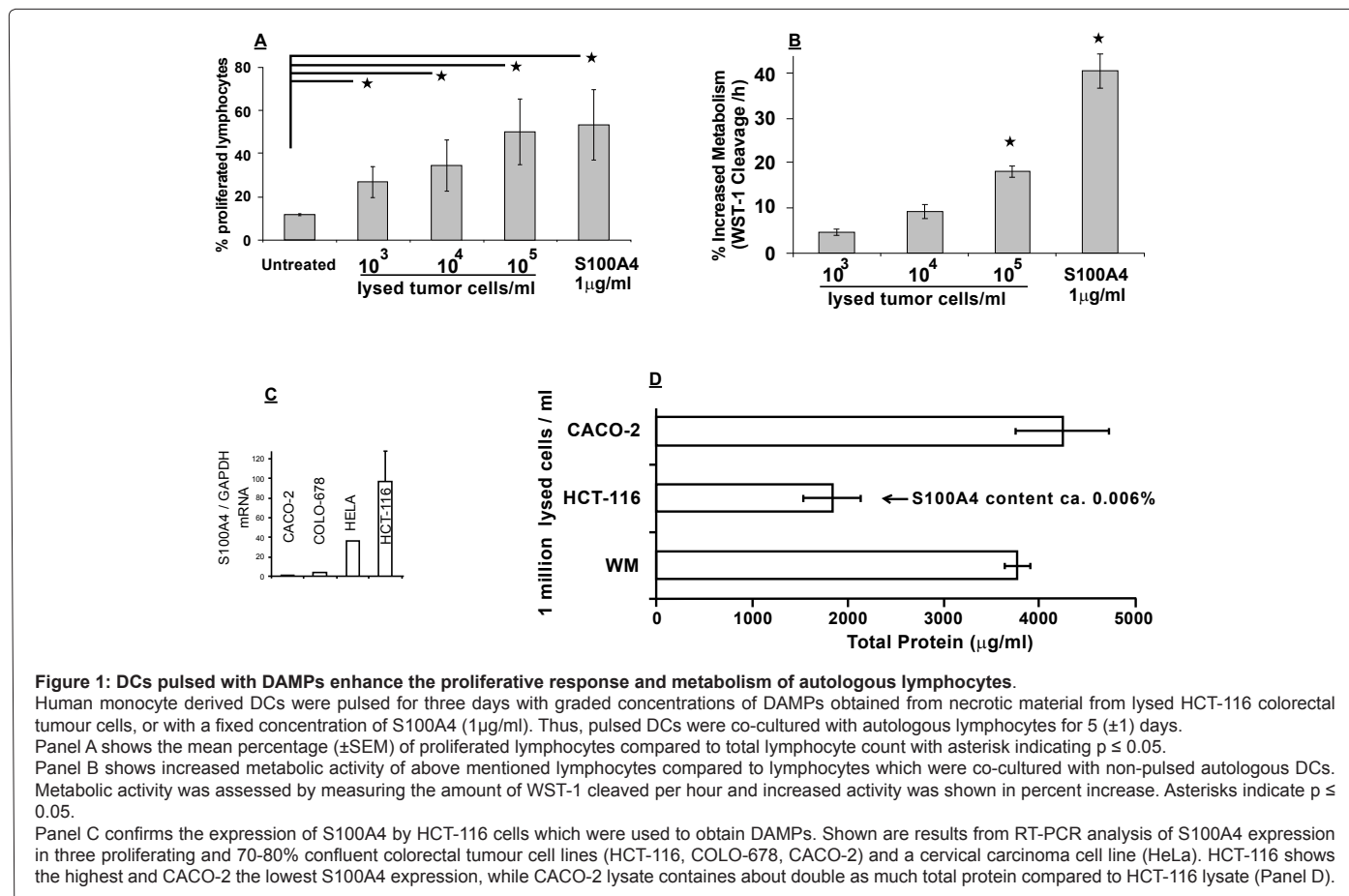
### Statistics

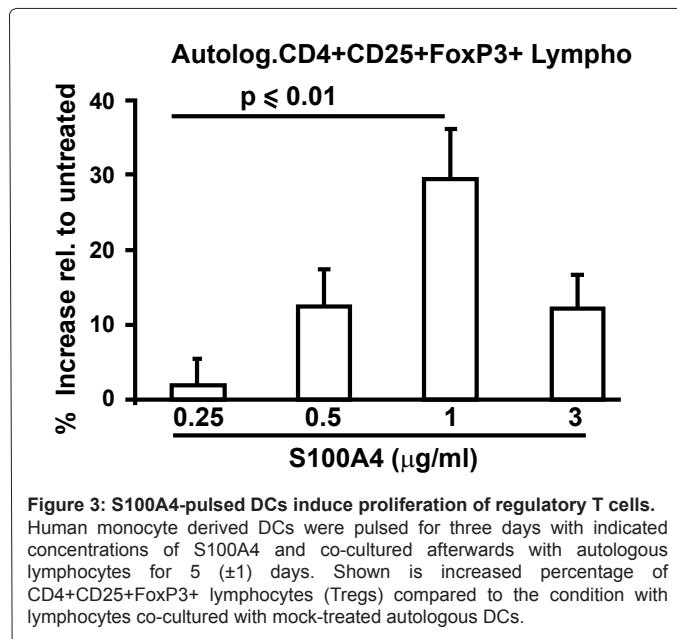
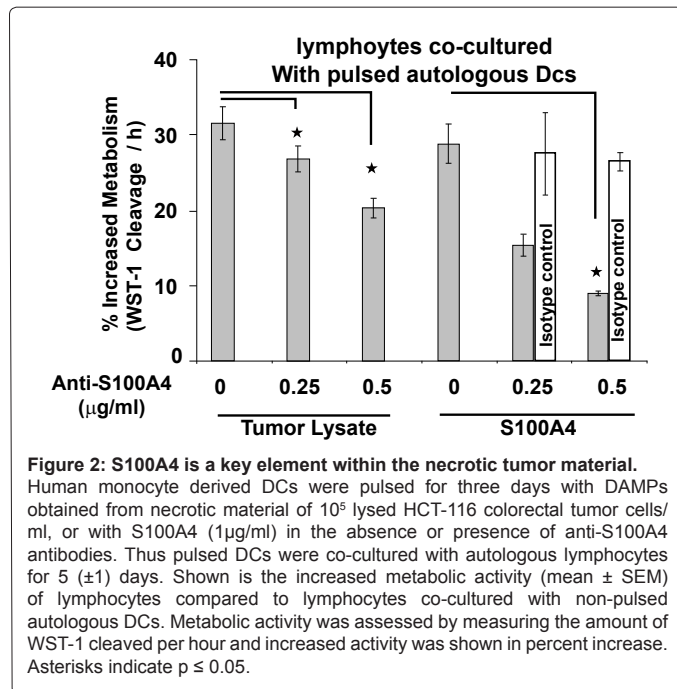
Student's t test for means (paired two samples) was used for calculating significance, and p-values equal or below 0.05 were considered as significant.

### Results

#### DCs pulsed with DAMPs enhance the proliferative response and metabolic activity of autologous lymphocytes

Based on our published results confirming that DAMPs from lysed colorectal tumour cells are capable of enhancing the expression of maturation markers CD40, CD80, and CD86 on human monocyte derived DCs [21], we now aimed to measure the ability of thus pulsed/matured DCs to induce lymphocyte proliferation. DCs which were pulsed for three days with indicated concentrations of necrotic material (DAMPs) induced a dose-dependent proliferative response of co-cultured autologous lymphocytes. S100A4 (1  $\mu$ g/ml)-a specific member of DAMPs which is thus also found within the tumour microenvironment-showed a similar stimulatory effect (Figure 1A). Performing WST-1 assays on the DC/Lymphocyte co-cultures we confirmed our flow cytometric results on the proliferative response of autologous lymphocytes, but also provided evidence for the dose-dependent enhanced metabolic activity of these lymphocytes which were co-cultured with autologous DC pulsed with graded concentrations of DAMPs, or alternatively pulsed with S100A4 at 1  $\mu$ g/ml (Figure 1B).





### S100A4 is expressed in tumour cells.

S100 proteins, including S100A4, belong to DAMP members constitutively expressed in almost all cells. Appreciating the fact that neoplastic cells may lose the expression of proteins which are found in healthy cells, we confirmed—as a proof of principle—the presence of S100A4 in the neoplastic colorectal cell lines, which were used in our assays to obtain DAMPs (Figure 1C).

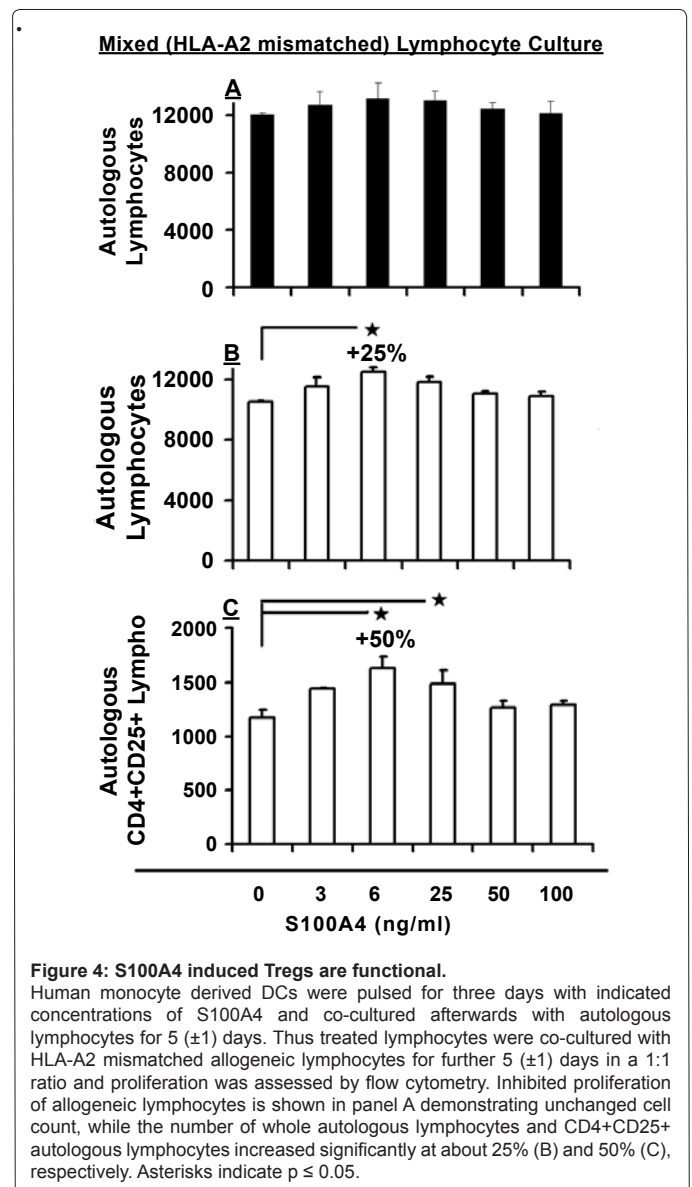
In order to better estimate the concentration of S100A4 needed to induce a similar effect as could be seen for DAMPs from lysed HCT-116 cells, we assessed the whole protein concentration of tumour lysates (Figure 1D). About 10<sup>6</sup> lysed HCT-116 cells contained approximately 100 ng S100A4 (data not shown).

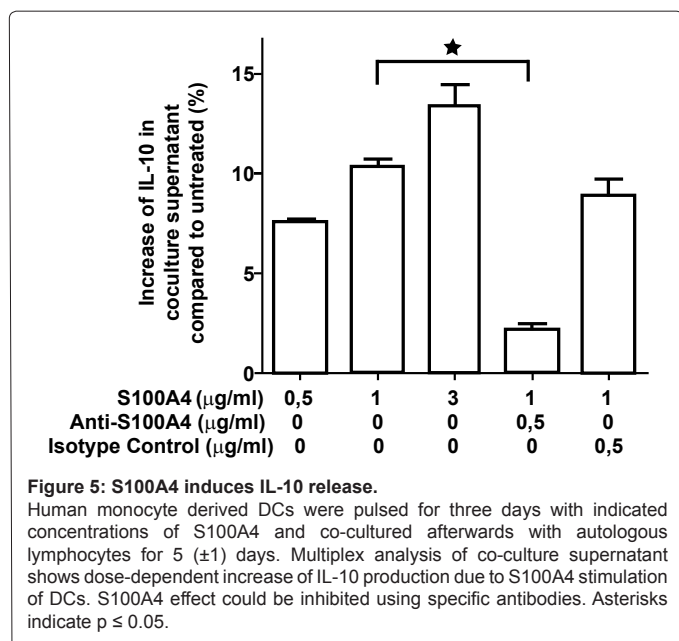
### S100A4 as a key element within the necrotic tumour material.

Given that DCs pulsed with S100A4 induced a strong proliferative lymphocyte response compared to the effect of whole tumour cell lysate (Figures 1A and 1B) it seemed conceivable that S100A4 plays a key role in the sum effect, thus inhibition assays with specific antibodies to S100A4 were performed. Anti-S100A4 inhibited dose-dependently the observed effect of tumour cell lysate and S100A4 protein on lymphocyte proliferation, whereas isotype control antibodies did not show any inhibitory effect ruling out the possibility of non-specific inhibition (Figure 2).

### S100A4-pulsed DCs induce proliferation of regulatory T cells

Flow cytometric assessment of markers specific for regulatory T cells confirmed the enhanced proliferation of CD4+CD25+FoxP3+ Tregs (Figure 3) when DCs were pulsed with S100A4 before co-culturing with autologous lymphocytes. In order to confirm the immunosuppressive capacity of these phenotypically described lymphocytes, functional assays were performed. For functional assays DCs were pulsed with





S100A4 at concentrations which are found within DAMPs obtained from  $3 \times 10^4$  to  $10^6$  lysed HCT-116 cells/ml, i.e. 3 to 100 ng S100A4/ml, pulsed DC were co-cultured according to previous experiments with autologous lymphocytes to generate Tregs; thus generated Tregs were co-incubated with allogeneic PBMC in a mixed lymphocyte culture demonstrating their inhibitory effect on the proliferation of allogeneic HLA-A2 mismatched lymphocytes (Figure 4A), while the number of (CD4+CD25+) Tregs increased significantly at about 50% (Figures 4B and 4C), the optimal effect could be observed when DCs were pulsed with 6 ng S100A4/ml. This observation was consistent with our previous data (Figures 1A and 1B) and (Figure 2) demonstrating the optimal effect when DCs were pulsed with lysates from  $10^5$  HCT-116 cells/ml, given that about 10 ng S100A4 is obtained from  $10^5$  lysed HCT-116 cells (data not shown).

Consistent with the immunosuppressive nature of S100A4 the supernatant of autologous DC-lymphocyte co-culture showed a dose-dependent increase of IL-10 (Figure 5), which is considered as an immunoregulatory/anti-inflammatory cytokine to facilitate the tissue-healing process in injuries and to repress proinflammatory responses, and thus limit unnecessary tissue disruptions caused by inflammation [24].

## Discussion

Current studies and therapeutic strategies addressing the immunosuppressive nature of tumour microenvironment focus on cytokines and chemokines released from a tumour or tumour-associated cells but under appreciated the biologic activity of DAMPs, and thus the impact of necrosis within the tumour milieu. We have recently shown that necrotic material (DAMPs) enhances the chemotaxis and proliferation of MSCs [20] which - given their strong immunoregulatory capacity-may interfere with effective cytotoxic immune response to the tumour. We now demonstrate here a further mechanism how DAMPs may impair anti-tumour immunity by inducing regulatory DCs and characterize now S100A4 as a specific DAMP member responsible for a major part of the observed effect. When applied extracellularly, S100A4 is able to promote metastasis, stimulate angiogenesis, induce cell motility and increase expression of

matrix metalloproteinases [25-28] partially explaining the association of S100A4 with poor prognosis of cancer patients [15-18]. The receptor for advanced glycation end products (RAGE) which is also expressed on monocytes and DCs is a putative receptor for S100A4 [15,29] but is not involved in S100A4 induced activation of NF- $\kappa$ B [30], thus further studies on S100A4 receptors and signaling pathways in DCs need to be performed in order to develop therapeutic strategies to abrogate the immunosuppressive effect of S100A4 on DC and thus on the adaptive immune response.

Of note, S100A4 belongs to oxidation sensitive proteins losing its stimulatory capacity following oxidation. Given the insufficient blood and oxygen supply to advanced tumour cells with subsequent reducing conditions prevailing within the tumour microenvironment, tumour-associated S100A4 is protected from inactivation due to oxidation. Interestingly, we could observe that the stimulatory effect of DAMPs on immunosuppressive MSCs was abrogated following oxidation of these factors [20]. Even though we did not perform any oxidation experiments on S100A4, these two notions suggest the therapeutic potential of strategies providing oxidizing conditions which could possibly abrogate the immunosuppressive activity of DAMPs in terms of DAMPs activity on tumour associated MSCs and DCs. Induction of reducing conditions may be considered on the other hand, when MSCs are used as novel cellular therapeutics for tissue regeneration and wound healing.

## Acknowledgement

The study was funded by German Society for Transfusion Medicine and Immunhematology (DGTI).

## References

- Ridgway D (2003) The first 1000 dendritic cell vaccinees. *Cancer Invest* 21: 873-886.
- Gilboa E (2007) DC-based cancer vaccines. *J Clin Invest* 117: 1195-1203.
- Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, et al. (2000) Clinical significance of defective dendritic cell differentiation in cancer. *Clin.Cancer Res* 6: 1755-1766.
- Vicari AP, Chiodoni C, Vaure C, Ait-Yahia S, Dercamp C, et al. (2002) Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. *J Exp Med* 196: 541-549.
- Vermi W, Bonecchi R, Facchetti F, Bianchi D, Sozzani S, et al. (2003) Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. *J Pathol* 200: 255-268.
- Proietti A, Ugolini C, Melillo RM, Crisman G, Elisei R, et al. (2011) Higher intratumoral expression of CD1a, tryptase, and CD68 in a follicular variant of papillary thyroid carcinoma compared to adenomas: correlation with clinical and pathological parameters. *Thyroid* 21: 1209-1215.
- Ugolini C, Basolo F, Proietti A, Vitti P, Elisei R, et al. (2007) Lymphocyte and immature dendritic cell infiltrates in differentiated, poorly differentiated, and undifferentiated thyroid carcinoma. *Thyroid* 17: 389-393.
- Feijoo E, Alfaro C, Mazzolini G, Serra P, Penuelas I, et al. (2005) Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8. *Int J Cancer* 116: 275-281.
- Gabrilovich DI, Ishida T, Nadaf S, Ohm JE, Carbone DP (1999) Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. *Clin Cancer Res* 5: 2963-2970.
- Morse MA, Hobeika AC, Osada T, Serra D, Niedzwiecki D, et al. (2008) Depletion of human regulatory T cells specifically enhances antigen-specific immune responses to cancer vaccines. *Blood* 112: 610-618.
- Dannull J, Nair S, Su Z, Boczkowski D, DeBeck C, et al. (2005) Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA

- encoding OX40 ligand. *Blood* 105: 3206-3213.
12. Vakkila J, Lotze MT (2004) Inflammation and necrosis promote tumour growth. *Nat Rev Immunol* 4: 641-648.
  13. Zeh HJ 3rd, Lotze MT (2005) Addicted to death: invasive cancer and the immune response to unscheduled cell death. *J Immunother* 28: 1-9.
  14. Lotfi R, Schrezenmeier H, Lotze MT (2009) Immunotherapy for cancer: promoting innate immunity. *Front Biosci* 14: 818-832.
  15. Boye K, Maelandsmo GM (2010) S100A4 and metastasis: a small actor playing many roles. *Am J Pathol* 176: 528-535.
  16. Mencia N, Selga E, Rico I, de Almagro MC, Villalobos X, et al. (2010) Overexpression of S100A4 in human cancer cell lines resistant to methotrexate. *BMC Cancer* 10: 250.
  17. Wang HY, Zhang JY, Cui JT, Tan XH, Li WM, et al. (2010) Expression status of S100A14 and S100A4 correlates with metastatic potential and clinical outcome in colorectal cancer after surgery. *Oncol Rep* 23: 45-52.
  18. Kang YG, Jung CK, Lee A, Kang WK, Oh ST, et al. (2012) Prognostic significance of S100A4 mRNA and protein expression in colorectal cancer. *J Surg Oncol* 105: 119-124.
  19. Lotfi R, Herzog GI, DeMarco RA, Beer-Stolz D, Lee JJ, et al. (2009) Eosinophils oxidize damage-associated molecular pattern molecules derived from stressed cells. *J Immunol* 183: 5023-5031.
  20. Lotfi R, Eisenbacher J, Solgi G, Fuchs K, Yildiz T, et al. (2011) Human mesenchymal stem cells respond to native but not oxidized damage associated molecular pattern molecules from necrotic (tumor) material. *Eur J Immunol* 41: 2021-2028.
  21. Herzog GI, Solgi G, Wiegmann DS, Nienhaus C, Schrezenmeier H, et al. (2011) Quality of tumor lysates used for pulsing dendritic cells is influenced by the method used to harvest adherent tumor cells. *BMC Res Notes* 4: 153.
  22. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, et al. (2007) Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449: 557-563.
  23. Lotfi R, Lotze MT (2008) Eosinophils induce DC maturation, regulating immunity. *J Leukoc Biol* 83: 456-460.
  24. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG (2011) Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol* 29: 71-109.
  25. Schmidt-Hansen B, Ormas D, Grigorian M, Klingelhofer J, Tulchinsky E, et al. (2004) Extracellular S100A4(mts1) stimulates invasive growth of mouse endothelial cells and modulates MMP-13 matrix metalloproteinase activity. *Oncogene* 23: 5487-5495.
  26. Belot N, Pochet R, Heizmann CW, Kiss R, Decaestecker C (2002) Extracellular S100A4 stimulates the migration rate of astrocytic tumor cells by modifying the organization of their actin cytoskeleton. *Biochim Biophys Acta* 1600: 74-83.
  27. Semov A, Moreno MJ, Onichtchenko A, Abulrob A, Ball M, et al. (2005) Metastasis-associated protein S100A4 induces angiogenesis through interaction with Annexin II and accelerated plasmin formation. *J Biol Chem* 280: 20833-20841.
  28. Schmidt-Hansen B, Klingelhofer J, Grum-Schwensen B, Christensen A, Andresen S, et al. (2004) Functional significance of metastasis-inducing S100A4(Mts1) in tumor-stroma interplay. *J Biol Chem* 279: 24498-24504.
  29. Heizmann CW, Fritz G, Schafer BW (2002) S100 proteins: structure, functions and pathology. *Front Biosci* 7: d1356-d1368.
  30. Grotterod I, Maelandsmo GM, Boye K (2010) Signal transduction mechanisms involved in S100A4-induced activation of the transcription factor NF-kappaB. *BMC Cancer* 10: 241.