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SHORT COMMUNICATION

Serum concentration of total soluble CD44 is elevated in smokers

DAVID A. SCOTT[1]*, PAULA Y. COWARD[1], RON F. WILSON[1], ROBIN N. POSTON[2], EDWARD W. ODELL[3] and RICHARD M. PALMER[4]

[1] Dental Clinical Research, Guy's, King's and St Thomas' Schools of Medicine, Dentistry and Biomedical Sciences, King's College London, Guy's Hospital, London, SE1 9RT, UK. e-mail: david.2.scott@kcl.ac.uk

[2] Department of Experimental Pathology, Guy's, King's and St Thomas' Schools of Medicine, Dentistry and Biomedical Sciences, King's College London, Guy's Hospital, London, SE1 9RT, UK

[3] Department of Oral Medicine and Pathology, Guy's, King's and St Thomas' Schools of Medicine, Dentistry and Biomedical Sciences, King's College London, Guy's Hospital, London, SE1 9RT, UK

[4] Department of Periodontology and Preventive Dentistry, Guy's, King's and St Thomas' Schools of Medicine, Dentistry and Biomedical Sciences, King's College London, Guy's Hospital, London, SE1 9RT, UK

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Soluble CD44 isoforms have been reported as markers of specific malignancies and inflammatory diseases. However, recent reports suggest tobacco smoking may lead to an elevation in the circulating concentration of specific CD44 variants. We, therefore, investigated the effect of smoking status on circulating levels of total sCD44. Total soluble CD44 was measured by enzyme-linked immunosorbent assay in the serum of two age- and gender-matched groups consisting of smokers ($n = 19$) and non-smokers ($n = 20$). Smoking status was confirmed by analysis of serum cotinine. The concentration of total sCD44 was found to be significantly elevated in smokers compared with non-smokers ($p = 0.025$). The observation that total sCD44 concentration is raised in smokers may have relevance to the aetiology of smoking-associated diseases. The effect of smoking on sCD44 concentrations should be considered when assessing the role of sCD44 as a marker of inflammatory disease, cancer, or other disease processes.

Keywords: tobacco smoking, CD44, cotinine, human.

Introduction

Human CD44 is a family of glycoproteins encoded by a single gene and CD44 is expressed on the surface of a wide range of cell types, such as fibroblasts, erythrocytes, myocytes and epithelial cells (Lazaar *et al*. 1990, Brennan *et al*. 1997, Ponta *et al*. 1998). CD44 is most studied for its roles in malignancy and lymphocyte homing, but is, in fact, a multifunctional adhesion molecule with important roles in the immune system and connective tissue maintenance. Serum levels of soluble CD44 (sCD44) have been shown to correlate with tumour metastasis in some cancers, including gastric and colon cancer (Guo *et al*. 1994, Lesley *et al*. 1997,

* *Corresponding author:* D. A. Scott, Dental Clinical Research, Guy's, King's and St Thomas' Schools of Medicine, Dentistry and Biomedical Sciences, Floor 17, Guy's Hospital Tower, King's College London, London, SE1 9RT, UK.

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Naot *et al*. 1997, Yamane *et al*. 1999). Soluble CD44 is also known to be raised in subjects with particular inflammatory conditions, such as rheumatoid arthritis (Haberhauer *et al*. 1997, Kittl *et al*. 1997a) and bronchitis (Kato *et al*. 1998). A wide range of CD44 ligands are known, including hyaluronate, fibronectin, serglycin, osteopontin and mucosal addressin (Naot *et al*. 1997, Ponta *et al*. 1998). Several reviews of CD44 structure and function have been published (Entwistle *et al*. 1996, Kincade *et al*. 1997, Lesley *et al*. 1997, Naot *et al*. 1997, Ponta *et al*. 1998).

There are at least 19 exons in the human CD44 gene encoding numerous CD44 isoforms by alternate splicing (Screaton *et al*. 1992, Ponta *et al*. 1998). However, all CD44 isoforms share the same N- and C-terminal sequences. The haematopoetic, or standard form, of CD44 (CD44H; CD44s) is the smallest isoform, with a molecular mass of 80–95 kDa (Screaton *et al*. 1992, Naot *et al*. 1997, Ponta *et al*. 1998). CD44s contains no variant exon-encoded peptide sequences (Screaton *et al*. 1992, Naot *et al*. 1997), therefore monoclonal antibodies recognizing CD44s (pan-C44 antibodies) can also bind all other CD44 isoforms.

Kittl *et al*. (1997b) recently observed a significant elevation in the mean concentration of two specific isoforms of sCD44 in smokers, compared with non-smokers, i.e. sCD44 containing the product of exon 5 (sCD44v5) and sCD44 containing the product of exon 6 (sCD44v6), during a study designed to examine these specific sCD44 isoforms as tumour markers in certain malignancies. Total sCD44, and other isoforms of CD44, were not considered by Kittl *et al*. (1997b). Of the multitude of CD44 variant proteins, expression of CD44s is predominant (Ponta *et al*. 1998). We, therefore, investigated the effect of smoking status on total sCD44 levels in a group of smoking and non-smoking subjects whose smoking status was validated by analysis of serum cotinine concentration.

Patients and methods

Patients

Thirty-nine white subjects, aged between 34 and 54 years (mean = 41.9, standard deviation = 4.8), were recruited from patients and staff at Guy's, King's and St Thomas' Dental Institute, King's College London. Written consent was obtained from each subject following an explanation of the study, approved by the Guy's Hospital Research Ethics Committee. A detailed medical history of each subject was obtained using a standard medical questionnaire. Subjects with any inflammatory condition, including dermatological conditions such as eczema, those taking anti-inflammatory drugs, including non-steroidal anti-inflammatory drugs, or subjects who did not meet the smoking status criteria were excluded from the study. Subjects with diabetes or who were pregnant were also excluded. Each subject enrolled into the study was in good general health.

Two groups, smokers ($n = 19$) and non-smokers ($n = 20$), were selected with the same number of males and females in each group, matched for gender and age. Smokers were initially defined as those who smoked more than 10 cigarettes per day for more than 1 year. Non-smokers were required not to have smoked at all during the previous 5-year period. The age ranges of the smoking and non-smoking groups were 35–49 years and 36–52 years, respectively. Mean ages are presented in table 1. Whole blood samples (5 ml) were collected by venepuncture, allowed to clot and serum obtained by centrifugation ($1300 \times g$, 10 min, room temperature). The serum samples were stored at –70 °C.

Table 1. Mean and (standard deviation) of clinical and laboratory data from smoking and non-smoking subjects.

	Smokers ($n = 19$)	Non-smokers ($n = 20$)
Age	41.8 (4.5)	42.1 (5.1)
Cigarette consumption per day	19.1 (6.0)	0.0 (0.0)*
Serum cotinine (ng ml^{-1})	290 (141)	0.6 (0.6)*
CD44 level (ng ml^{-1})	250 (30)	227 (31)**
Difference in CD44 level (ng ml^{-1}) [95% +23 [+7 to +43]** confidence interval]		

* $p < 0.001$; ** $p = 0.025$.

Measurement of serum levels of sCD44 and cotinine

Assay of sCD44 was performed using a commercially available ELISA kit (R and D Systems, UK), according to the manufacturer's instructions. Each assay was performed in duplicate. Optical density was measured at 450 nm using a Dynatech MR 700 automated microplate reader. The concentration of sCD44 was calculated from a calibration curve using duplicate standard concentrations of CD44s.

Serum cotinine levels were measured by ABS Laboratories, Medical Toxicology Unit, UK. A smoker can be reliably differentiated from a non-smoker by serum cotinine levels using an optimal cut-off value of 13.7 ng ml^{-1} cotinine (Russell *et al*. 1980, Jarvis *et al*. 1987).

Statistical evaluation

Statistical analysis was carried out using SPSS for Unix, release 6.1 and Genstat 5, release 3.1. *T*-tests were used for within group comparisons. One way analysis of variance was used for comparison between groups.

Results

The smoking status of all subjects in the study was confirmed by serum cotinine analysis, presented in table 1. Interestingly, the two smoking subjects representing the extreme values in the cotinine range (82.8–688.2 ng ml^{-1}) both reported that they smoked 20 cigarettes per day. The serum concentration of total sCD44 was significantly higher in smokers, compared with non-smokers (see table 1). A higher concentration of total sCD44 was observed in 84% (16/19) of the smokers, compared with specific age and gender matched non-smoking pairs. Although there was a significant elevation in the sCD44 levels of those individuals defined as smokers by serum cotinine analysis, there was no significant dose-dependent correlation between serum cotinine load and sCD44 levels in this study ($p = 0.157$).

Discussion

Cotinine is the major catabolite of nicotine and is thus a specific and accurate biomarker of current smoking status (Russell *et al*. 1980, Jarvis *et al*. 1987). Self-reported smoking habits may be unreliable and analysis of dose-dependent relationships may be further complicated because of variable factors in the smoking habits of individuals, such as frequency and depth of inhalation. This is supported by the fact that in this study the smoker with the lowest serum cotinine level (82.8 ng ml^{-1}) and the highest (688.2 ng ml^{-1}) both reported that they smoked the same number of cigarettes daily. We did not observe any statistically significant correlation between sCD44 and serum cotinine levels, although it may be prudent to revisit this issue using larger numbers of smoking subjects.

The observation that smoking is associated with an elevated concentration of

total sCD44 levels is of great interest. Although it is not known if a mean increase in total sCD44 concentration of 11% in smokers, compared with non-smokers, is clinically important, the difference is statistically significant. Elevated levels of sCD44 have been reported as markers of several diseases (Guo *et al*. 1994, Haberhauer *et al*. 1997, Kittl *et al*. 1997a, Lesley *et al*. 1997, Naot *et al*. 1997, Ristamaki *et al*. 1997, Kato *et al*. 1998, Yamane *et al*. 1999) and the results of the present study would suggest that great care should be taken in the interpretation of clinical studies aiming to assess the role of soluble forms of CD44 in disease processes and immune function when smoking has not been accounted for in the study design.

Although we have shown a small, but significant, elevation of total sCD44, Kittl *et al*. (1997b) have previously reported that the mean concentration of sCD44v5 in smokers is double that of non-smokers. Therefore, it is probable that the raised sCD44 level observed in smokers will be constituted of molecules of specific sCD44 isoforms and is not the result of a blanket effect of tobacco smoking on all CD44 gene products. Many CD44 isoforms are tissue-specific but the specific variant isoform(s) of sCD44 that are elevated due to the smoking experience remain to be clarified. Thus, further investigations into the character, and source, of elevated sCD44 molecules may shed light on the relevance of raised sCD44 in the context of the aetiology of smoking-associated diseases.

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