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CASE REPORT

Granulocytosis and thrombocytosis in renal cell carcinoma: a pro-inflammatory cytokine response originating in the tumour

A.P. van Rossum[1], L.Th. Vlasveld[2*], I.N. Vlasveld[3], P.M. Jansen[4], W.A. Dik[5], H. Hooijkaas[5], A. Castel[1]

[1]Laboratory for Clinical Chemistry and Haematology, [2]Department of Internal Medicine, Bronovo Hospital, the Hague, the Netherlands, [3]Medical student, University of Leiden, Leiden, the Netherlands, [4]Department of Pathology, Leiden University Medical Centre, Leiden, the Netherlands, [5]Department of Immunology, Erasmus Medical Centre, University Medical Centre, Rotterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)70-312 41 41, e-mail: tomvlasveld@hotmail.com

ABSTRACT

Background: In up to 20% of patients with renal cell cancer (RCC) an inflammatory response consisting of low-grade fever, weight loss and an elevated ESR and CRP may occur with modest granulocytosis and thrombocytosis. Clinical and experimental data suggest a pathogenic role for tumour-derived cytokine production, especially interleukin-6.

Case report: A 79-year-old female with RCC presented with low-grade fever, weight loss and overt granulocytosis and thrombocytosis. Radiological examination revealed a right-sided renal tumour. During nephrectomy a gradient between the IL-6 levels in the renal artery and vein was demonstrated, providing direct evidence for *in vivo* production of IL-6 by the tumour affected kidney, which was confirmed by the demonstration of IL-6 in the tumour cells by immunohistochemical staining and in the supernatant of the homogenised tumour. Cytogenetic examination revealed complex abnormalities including a gain of chromosome 7. In addition we demonstrated production of IL-1α, IL-1β, IL-8 and ICAM-1 in the tumour with systemic elevated levels of IL-6 and IL-8 with secondary increased serum G-CSF and TPO levels.

Conclusion: We have provided direct evidence for the production of pro-inflammatory cytokines by renal cancer cells in a patient with RCC and a profound inflammatory response, with a central role of IL-6, probably due to a gain of chromosome 7. The extreme granulocytosis and thrombocytosis may have resulted from the secondary systemic production of G-CSF and TPO.

KEYWORDS

Cytokines, inflammation, interleukin-1, interleukin-6, renal cell carcinoma, trombocytosis

INTRODUCTION

Ten to forty percent of patients with renal cell cancer (RCC) experience paraneoplastic phenomena during the course of the disease.[1] The symptoms may result from the production of humoral factors by the tumour or by adjacent tissue in response to the tumour or via immune modulation. Erythrocytosis is the most well-known paraneoplastic haematological event. Although two-thirds of the renal tumours produce erythropoietin, erythrocytosis is reported in 1 to 8% of the patients.[1] Overt granulocytosis (>50/nl) has occasionally been reported in patients with G-CSF or GM-CSF producing RCC.[2-4] Modest granulocytosis and thrombocytosis occurs in up to 20% of the patients and is thought to be part of a systemic inflammatory response since it is usually associated with anaemia, fever, weight loss, and increased serum levels of CRP and interleukin-6.[5] Here we report a patient with RCC, who presented with fever, weight loss and marked granulocytosis and thrombocytosis. We performed extensive cytokine profiling of the tumour and normal renal tissue as well as in blood samples obtained from peripheral blood, renal vein and renal artery. We demonstrated production of IL-1α, IL-1β, IL-6, IL-8 and ICAM-1 in the tumour with systemic elevated levels of IL-6 and IL-8 with secondary increased serum levels of granulocyte-colony stimulating factor (G-CSF) and thrombopoietin (TPO).

CASE REPORT

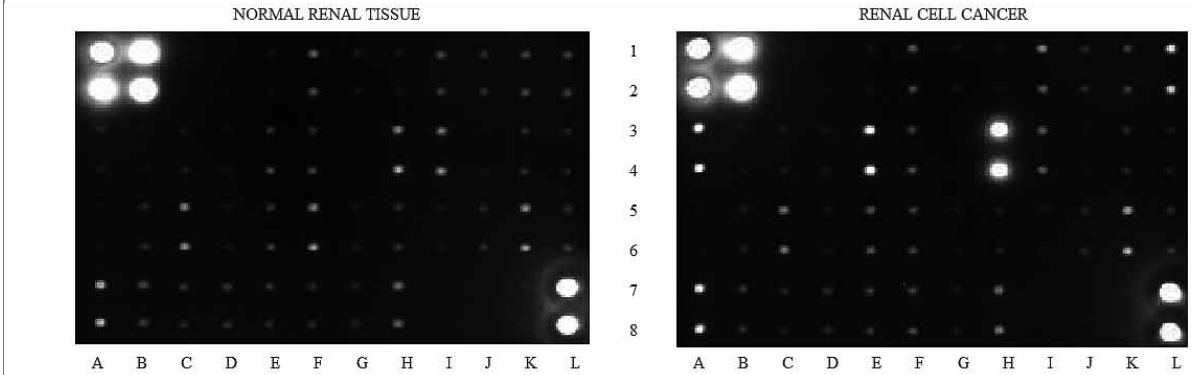
A 79-year-old female presented with low back pain, low-grade fever and 6 kg weight loss in three months' time. Physical examination was unremarkable. Laboratory analysis showed an ESR of 123 mm (RR <30) and serum CRP of >200 mg/l (RR <8). Haemoglobin level was 6.1 mmol/l (RR 7.4 to 10.2), granulocytes were 40/nl (RR 1.5 to 7.0) with 665/nl thrombocytes (RR 150 to 400). An extensive biochemical profile showed a serum albumin level of 31 g/l (RR 34 to 48) but was otherwise normal. Computed tomography showed a right-sided renal tumour with extension into the pyelum without distant metastases. During radical nephrectomy samples of renal artery and vein were collected and GM-CSF, G-CSF, IL-1α, IL-6, IL-8 and TPO were determined by conventional ELISA (BioSource, CA, USA) or by human inflammation Th1/Th2 cytometic bead array (CBA; BD Biosciences, San Jose, USA) according to the manufacturer's guidelines. As shown in *table 1*, a 1.5 gradient was detected for IL-6 between the renal vein and artery, indicating production of IL-6 by the tumour infiltrated kidney. The systemic level, as reflected by the concentration in the renal artery, of IL-6 was increased to 6.2 pg/ml (RR 0.2 to 4.6), and of IL-8 was increased to at least 80 pg/ml (RR <10) while the levels of GM-CSF, G-CSF, IL-1α and TPO were within the normal range. Pathological examination showed a renal cell cancer with extensive sarcomatoid differentiation (Fuhrman grade 4) with a diameter of 8 cm with regional lymph node metastases. In the tumour neutrophilic infiltration was present. Cytogenetic examination showed complex abnormalities: 47, XX, + 18 (two cells), 48, XX, +9, +10 (one cell), 92, XXXX (three cells), 77-93, XXXX, +18, +7, -9mar[cp4] (four cells) and 47, XX, +10 (one cell). Sample suspensions of the tumour and the normal adjacent renal tissue were made by homogenising 4 mm^3 tissue in 2 ml PBS using a Potter's homogeniser. Cytokine

Table 1. *Results of cytokine profiling*

Cytokine	Tumour/normal tissue	Renal vein/artery
IL-1α	7.5#*	1.0#
IL-1β	76.3*$	n.d.
IL-6	5.9#*$	1.5#$
IL-6sR	1.8*	n.d.
IL-8	11.5#*$	0.9#$
G-CSF	n.d. (due to inconsistent results)	1.2#$
GM-CSF	0.5#*	0.9#
TPO	1.3#	1.1#
ICAM-1	1.6*	n.d.

The ratio is expressed by means of combining the results of the conventional ELISA (#), the Quantibody Human Inflammation Array 3 (*) and/or the human inflammation TH1/Th2 cytometic bead array ($). n.d. = not determined.

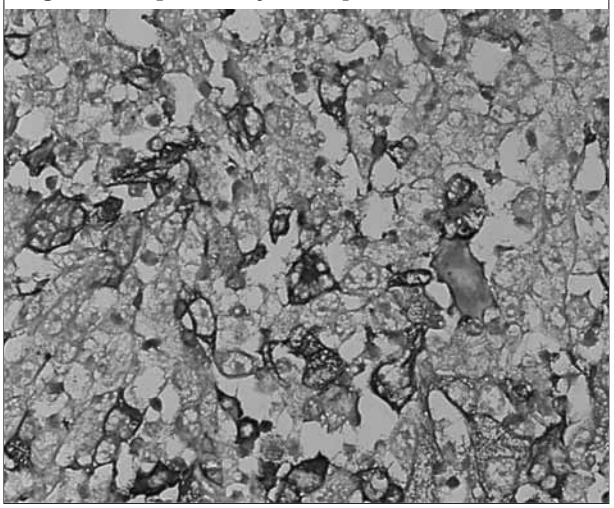
Figure 1. *RayBio human inflammation antibody array 3 in comparison with the normal renal tissue the renal cell cancer shows increased expression of IL-1α, IL-1β, IL-6, IL-8, ICAM-1 and CCL-5*



	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	Eotaxin	Eotaxin-2	G-CSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
2	POS	POS	NEG	NEG	Eotaxin	Eotaxin-2	G-CSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
3	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6sR	IL-7	IL-8	IL-10	IL-11	IL-12p40	IL-12p70
4	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6sR	IL-7	IL-8	IL-10	IL-11	IL-12p40	IL-12p70
5	IL-13	IL-15	IL-16	IL-17	IP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
6	IL-13	IL-15	IL-16	IL-17	IP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
7	CCL-5	TGF-β1	TNF-α	TNF-β	sTNF RI	sTNF RII	PDGF-BB	TIMP-2	Blank	Blank	Neg	Pos
8	CCL-5	TGF-β1	TNF-α	TNF-β	sTNF RI	sTNF RII	PDGF-BB	TIMP-2	Blank	Blank	Neg	Pos

profiling of the supernatant of these samples was done by the above-mentioned ELISA and bead array as well as by Quantibody Human Inflammation Array 3 (RayBiotech, Norcross, GA, USA). As indicated in *table 1* and *figure 1*, concentrations of IL-1α, IL-1β, IL-6, IL-8 and ICAM-1 in the supernatant of the tumour tissue were higher compared with normal renal tissue. There was no evidence for production of GM-CSF, G-CSF, TPO and a variety of other cytokines (*figure 1* and *table 1*). Immunohistochemical staining showed the presence of IL-6 in the cytoplasma of the renal tumour cells (*figure 2*) while the staining for IL-8 was more prominent in the tumour infiltrating granulocytes. After nephrectomy the granulocyte and platelet counts nearly normalised. However, the patient's condition deteriorated due to rapidly progressive metastatic disease and she died 54 days after nephrectomy. At that time the granulocytes were 72.2/nl and thrombocyte count 810/nl. There were increased serum levels of G-CSF (182 pg/ml [RR 7.8 to 38.9]), IL-6 (23.9 pg/ml), IL-8 (80 pg/ml) and TPO (105 U/ml [RR 4-32]), while the serum concentrations of IL-1α and GM-CSF were within the normal range.

Figure 2. *Expression of IL-6 by the renal tumour*



DISCUSSION

This patient with RCC presented with systemic inflammatory response and profound granulocytosis and thrombocytosis. We have provided evidence for production of IL-6 by the renal cell tumour by the presence of an IL-6 level gradient of 1.5 between the renal vein and artery, a high concentration of IL-6 in the supernatant of the tumour compared with that of normal adjacent renal tissue and the demonstration of IL-6 in the cytoplasm of the tumour cells by immunohistochemical staining. In addition, cytokine profiling of the supernatant of the tumour revealed an indication for production of IL-1α, IL1-β, IL-8, and ICAM-1 by the tumour affected kidney. This resulted in elevated systemic levels of IL-6 and IL-8.

IL-6, encoded by a gene located on chromosome 7p21-p14, is a pleiotropic cytokine which promotes the growth and action of cytotoxic T cells, acts synergistically with IL-3 in haematopoiesis and induces differentiation of various cells including megakaryocytes. IL-6 has a central role in the acute-phase response by stimulating hepatocytes to produce acute-phase proteins such as CRP and fibrinogen and suppress albumin production. It is produced primarily by lymphocytes and monocytes, but also by numerous other tissues, including a variety of tumours.[6] Normal renal cells may express low amounts IL-6 which may be upregulated by exogenous stimuli.[7] In the vast majority of renal cancer cell lines as well as in freshly isolated renal cell cancer specimens IL-6 is the predominantly produced cytokine.[8,9] Furthermore, serum IL-6 levels are elevated in many patients with RCC. In most studies there is a relation between the serum IL-6 level and the systemic inflammatory response reflected by fever, weight loss and elevated CRP or ESR.[5,10-11] In approximately 15 to 25% of these patients mild granulocytosis and/or thrombocytosis may occur.[12] Thrombocytosis, granulocytosis and elevated serum IL-6 levels are usually associated with a poor prognosis.[12,13] Since IL-6 is not expressed in all cases of RCC, additional factors to induce IL-6 production must be present. Increased IL-6 production has been demonstrated in renal cell tumours with mutated p53[14] and in renal cell cancer with gain of an additional chromosome 7 as in our patient.[6] A variety of cytogenetic abnormalities, such as gain of chromosome 7, may occur in the different RCC histological subtypes, including those with sarcomatoid features, with diagnostic and prognostic implications.[15,16] Once expressed, IL-6 acts as an autocrine factor.[6]

Since we performed the cytokine profile in the supernatant of the homogenised tumour it is questionable whether production of IL-1α, IL-1β, IL-8 and ICAM-1 originated from the tumour or from infiltrating neutrophils. It may be hypothesised that tumour-derived IL-1α, IL-1β, IL-8 and ICAM-1 induce migration and attraction of the neutrophils. Furthermore, the produced IL-6 and IL-8 prohibit the infiltration of (tumour-specific) cytotoxic T-cells, which may explain the lack of tumour infiltrating lymphocytes and may contribute to the aggressive course of the disease in our patient.[17] Otherwise, it may be assumed that the invading neurophils are responsible for additional cytokine production, such as IL-8, as indicated by the immunohistochemical staining of IL-8 in both the tumour cells and neutrophils. Neutrophils are able to produce IL-8; however, they cannot produce IL-1 and IL-6, neither constitutively nor post-stimulation.[18] So both the tumour cells and the

invading neutrophils may be responsible for different parts of the systemic inflammatory response.

Maturating neutrophils and platelets bind G-CSF and TPO respectively which may result in a reciprocal relationship between the cytokines and the circulating cells.[19] The fact that serum G-CSF and TPO is increased during extreme granulocytosis and thrombocytosis indicates excessive production of these cytokines. We found no evidence for production of G-CSF or TPO within the tumour.

It is very likely that the increased systemic level of G-CSF may be the result of the tumour-derived production of IL-1, while the increased TPO level is secondary to the production of IL-6 by the tumour.

CONCLUSION

We report a patient with RCC with low-grade fever, weight loss and elevated ESR and CRP resulting from the release of pro-inflammatory cytokines within the tumour affected kidney with a central role for tumour-derived IL-6, probably due to a gain of chromosome 7. The extreme granulocytosis and thrombocytosis may have resulted from the secondary systemic production of G-CSF and TPO.

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