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Biological Response to Intrahepatic Adoptive Immunotherapy with Autologous Interferon Activated Macrophages

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BIOLOGICAL THERAPY has recently emerged as a treatment for cancer. Within the host defense system against the development and spread of malignant tumours, the mononuclear phagocytic cells play an important role. Early clinical trials using monocytes or macrophages (MAC) for adoptive transfer in order to correct for defective generation of competent effector cells in patients with cancer have been published [1–3]. Here we report on the biological effects of adoptive immunotherapy with autologous MAC by hepatic artery infusion in 7 patients with metastatic liver disease.

Mononuclear cells were obtained on 3 consecutive days by cytapheresis and cultured in supplemented medium with 2% autologous serum. On day 6 of culture, cells were activated with interferon-gamma 200 IU/ml (Bioferon, Laupheim, FRG). The following day, cells were harvested, purified by centrifugal elutriation and administered into the hepatic artery.

Clinical side-effects were usually mild and disappeared in all cases within 24 h after therapy. Fever (>37.5°C) occurred in 20 of 35 therapies with a maximum temperature of 39.4°C observed in a patient receiving 0.81×10^8 MAC (Table 1). Temperature elevation reached a maximum at 4–8 h after therapy and returned to normal values within 24 h. Other adverse events were nausea, dizziness, headache and general malaise.

A remarkable change concerning the coagulation parameters was detected within the first 4 h after treatment (Table 1). In one third of therapies, an increase of thrombin–antithrombin (TAT)-complexes was noted, corresponding with the detection of circulating fibrin monomers and indicating the induction of the coagulation cascade. However, there was no evidence of disseminated intravascular coagulation or thrombembolic complications with clotting time, reptilase, antithrombin (AT) III and platelet counts remaining unchanged. This is in accordance with our observation using intravenous and intraperitoneal treatment [1] and with the report of Wiesel and colleagues [4]. Normal levels of coagulation inhibitors, such as AT III, protein C and protein S, were apparently sufficient to maintain the haemostatic balance: no patient suffered from haemorrhagic or thrombotic events. Elevated levels of circulating C-reactive protein (CRP) were detected in all patients. A rise of CRP was not only seen within hours after therapy, but also consisted of successive increases in serum levels during therapy cycles (9 or 12 therapy cycles).

Table 1. Biological response to adoptive macrophage therapy

MAC infused (10^9)	MAC infusions (*n*)	Fever* (%)	CRP† (%)	TAT‡ (%)
<1	6	50§	17	17
1–5	14	79	50	29
6–10	12	42	58	50
>10	4	50	100	n.a.

* Body temperature higher than 37.5°C. † Increase of CRP of more than 0.5 mg/dl within 24 h after therapy. ‡ Increase of TAT-complexes of more than 3 ng/ml with 4 h after therapy. § Data are expressed as percentage of total numbers of MAC therapies. n.a., not available.

No response was seen with all 7 patients showing progressive disease 4 weeks after therapy. However, our results show that regional intrahepatic adoptive immunotherapy with autologous *ex vivo* generated MAC is well tolerated without major side-effects. A profound biological response is elicited in the autologous recipient. Thus, regional adoptive immunotherapy might be able to build up a potent cytotoxic cell infiltrate which could be triggered within the patient by exogenous stimuli such as endotoxin, cytokines or other MAC activators.

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Determination of Oestrogen Receptor by Enzyme Immunoassay

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THE ARTICLE by Romain and associates [1] describes a number of technical difficulties that could account for high variability in

the measurement of oestrogen receptor values in breast cancer biopsies between different laboratories. The article particularly cautions on the various conformational changes that may occur in the receptor and subsequently influence their recognition by the enyzme immunoassay kit distributed by the Abbott Laboratories (Chicago, Illinois).

I would like to bring to the attention of your readers a few additional variables that we found to critically influence the enzyme immunoassay in a study that was published by us earlier [2]. Our study [2] concurs with the study of Romain and associates [1] with regard to the conformational changes of the oestrogen receptor and the ability to be recognised by the antibody. We also found that unliganded receptor was recognised differently by the antibody assay than either the oestradiol or the diethylstilbestrol bound receptor. Therefore, biopsies of patients exposed to different levels of circulating hormones or other chemicals that recognise oestrogen receptor could give 'false values' by the enzyme immunoassay.

An important observation was made by us regarding the conformation of the receptor that was recognised by the enzyme immunoassay kit. It was demonstrated [2] that only the non-proteolysed 4S form of the receptor (i.e. the dissociated 8S form), and not the 8S form of the receptor, was recognised by the antibody in the kit. Sample dilution buffer provided in the enzyme immunoassay kit contains components (KCl) to cause such a dissociation [2]. Therefore, in-house buffers that prevent the 8S→4S conversion would prevent the recognition of the oestrogen receptor with the antibody. In addition, we found that the proteolysed form of the receptor that retained the ligand binding site for oestradiol was also not recognised by the kit. Since breast tumours contain varying degrees of proteases, some of which may cause an 8S→4S proteolytic conversion, the use of protease inhibitors should also be considered in quality control trials.

Romain and colleagues [1] have raised an important issue related to the need for quality control of enzyme immunoassay measurements of oestrogen receptor in different laboratories. Every effort should be made to minimise the inter- and intra-laboratory measurements of the oestrogen receptor and to maximise the conditions for the most favourable conformational form of the oestrogen receptor to be recognised by the enzyme immunoassay kit. Therefore, it is essential for there to be a consensus with regard to the buffers that are to be utilised with the enzyme immunoassay kit in the measurement of oestrogen receptor for clinical use.

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Mutational Analysis of the *MCC* Gene by Single-strand Conformational Polymorphism Analysis

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A REGION on chromosome 5q21 is commonly deleted in sporadic colorectal carcinomas, and has been shown to contain the gene responsible for familial adenomatous polyposis (FAP), an inherited form of colorectal cancer [1, 2]. Several genes have been mapped to this region including both *APC* and *MCC* [3–6]. *APC* has since been shown to be the gene responsible for FPC, and is also mutated somatically in the majority of sporadic colon cancers. The role of *MCC* is, however, still uncertain. No mutations of *MCC* have been found in FAP patients, and only six somatic mutations in sporadic tumours have been reported [6, 7].

This study screened the 17 exons of the coding region of the *MCC* gene for mutations in a small subset of clinically important colorectal tumours from 5 non-polyposis patients who presented with the disease at a relatively young age (Table 1). We considered it possible that defects in *MCC* might be important in colorectal tumorigenesis in this subset of younger non-polyposis patients whilst having little input on the development of sporadic tumours in older patients. Mutational analysis was performed by the technique of single-strand conformational polymorphism analysis after amplification by the polymerase chain reaction (PCR-SSCP) on normal and tumour tissue as described previously [6] using PCR primers previously published [8]. We used DNA from these tumours previously grown as xenografts in SCID mice to eliminate the presence of any normal stromal elements which may have rendered mutational analysis difficult.

The method detected abnormalities in all 5 previously reported cases with *MCC* mutation. No mutations, however, were found in the coding region of *MCC* in any of the 5 young patients, although several polymorphisms were noted. A deletion polymorphism in exon 10 and a C-T conservative base change at codon 708 of exon 15 have been reported previously [9]. A novel polymorphism was observed in 2 of the 5 patients. Sequencing of these samples revealed a two base pair change (GC-TG) within intron 13 immediately prior to exon 14. This polymorphism appeared to be linked to the polymorphism in exon 15 and this was confirmed by extending the study of these two exons to an additional 19 constitutional DNA samples from young non-polyposis colorectal cancer patients and 18 normal control patients. The allele frequency of the novel polymorphism (B1 in Table 1) was 83% compared to the published sequence of

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