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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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1. Andreesen R, Scheibenbogen C, Brugger W, Krause S, Meerpohl HG, Leser HG, Engler H, Löhr GW. Adoptive transfer of tumor cytotoxic macrophages generated in vitro from circulating blood monocytes: a new approach to cancer immunotherapy. *Cancer Res* 1990, **50**, 7450–7456.
2. Lopez M, Fechtenbaum J, David B, Marinache C, Chokri M, Canepa S, De Gramont A, Louvet C, Gorin I, Mortel O, Bartoleyns J. Adoptive immunotherapy with activated macrophages grown in vitro from blood monocytes in cancer patients: a pilot study. *J Immunother* 1992, **11**, 209–217.
3. Andreesen R, Hennemann B. Adoptive immunotherapy with autologous macrophages: current status and future perspectives. *Pathobiology* 1991, **59**, 259–263.
4. Wiesel ML, Faradji A, Grunebaum L, *et al.* Hemostatic changes in human adoptive immunotherapy with activated blood monocytes or derived macrophages. *Ann Hematol* 1992, **65**, 75–78.

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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