Adaptive immunotherapy of cancer using monocyte-derived macrophages: rationale, current status, and perspectives

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Abstract: Adoptive transfer of host defense cells may be able to correct an otherwise defective generation of competent immune cells in patients with cancer. Ex vivo-grown cytotoxic macrophages (MAC) able to recognize and destroy tumor cells but not normal cells are effective in murine models of metastasizing tumors. After the development of large-scale technology to generate MAC in vitro from blood monocytes (MO), clinical trials in cancer patients have proven the feasibility and safety of infusing 3 x 10⁹ autologous MO-derived MAC activated by interferon-γ or lipopolysaccharide. Various modalities of adaptive immunotherapy with human MAC have been realized: routes of application used were intravenous, intraperitoneal, intra-pleural, and through selective hepatic artery perfusion. In addition, MAC have been generated from MO collected after granulocyte-macrophage colony-stimulating factor treatment in vivo. Biodistribution studies using ¹¹¹indium-labeled cells have revealed localization of MAC to sites of bulk tumor growth on regional infusion as well as to liver metastases on systemic application. Malignant ascites disappeared in about 50% of patients after intraperitoneal treatment, yet no other evidence of therapeutic efficacy of MAC could be demonstrated. Further advances of adoptive transfer of MO-derived cells are developed with emphasis on the generation of antigen-presenting cells primed in vitro with tumor cells or specific peptides. J. Leukoc. Biol. 64: 419-426; 1998.

Key Words: cellular vaccine · tumor cytotoxicity · dendritic cells

INTRODUCTION

Cellular immunotherapies using lymphoid effector cells have been successfully applied in the treatment of patients with relapsed chronic and acute myeloid leukemias [1, 2], with Epstein-Barr virus-associated lymphoma [3, 4], and also to provide anti-viral immunity [5, 6]. Cells of the mononuclear phagocyte system (MPS) not only are major players in natural immunity to microbial infections but also participate in host defense against malignant tumors, both activities being defined as innate resistance [7]. The MPS is ubiquitously found in all tissues and body cavities. It is comprised of fixed cells residing as organ-specific populations and of a mobile pool of circulating blood monocytes (MO) that are ready to invade infected areas in response to inflammatory stimuli. MO also represent precursor cells that on demand, as well as in steady-state conditions, serve as a source for the constant renewal of tissue macrophages (MAC). Although interacting with and being regulated by the specific T and B cell system, MO/MAC have powerful constitutive antimicrobial and antineoplastic properties even in the absence of specific immunity. They seem to be able to distinguish malignancy [8] and attack neoplastic cells through contact-dependent and cytotoxicity-mediated mechanisms [9], but the precise molecular events leading to target cell death still remain elusive.

MACROPHAGE INTERACTION WITH MALIGNANT TUMORS

Although cells of the MPS interact with malignant tumors in multiple ways, MAC obviously play an ambiguous role [10]. Whereas most malignant tumors contain numerous MAC as a major constituent of their leukocytic infiltrate, at least for established solid tumors there is little evidence that these tumor-associated macrophages (TAM) are detrimental to the tumor but rather seem to coexist with the malignant cells in a symbiotic manner. MAC and their soluble products may even sustain tumor development and progression, e.g., by directly stimulating cell growth [11], inducing neoangiogenesis [12], tissue destruction, and somatic hybridization of tumor cells with the inflammatory macrophage itself [13]. The latter event is postulated to result in highly metastatic variants of the original clone and to contribute to tumor cell spread away from the site of its original transformation. Paraneoplastic symptoms of a malignant tumor like the wasting syndrome, fever, and neutrophilia may be mediated in part by their MAC content [14]. Clinical data on the correlation of poor prognosis linked to the extent of MAC infiltrate in breast cancer patients [15] as well as early reports on the differential cytotoxicity of MAC isolated from regressing or progressing tumors [16] support this hypothesis.

To understand this ambiguity it may be crucial to analyze the

Abbreviations: MPS, mononuclear phagocyte system; MO, monocytes; MAC, macrophages; TAM, tumor-associated macrophages; IFN-γ, interferon-γ; GM-CSF, granulocyte-macrophage colony-stimulating factor.

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differential ontogeny of the TAM MAC: the circulating blood MO are the precursors of both the symbiotic and the cytotoxic host macrophage. On their emigration from the vasculature into tissues they undergo differentiative events that are controlled by the specific microenvironment they enter. Here, factors derived from various physiological as well as reactive tissue constituents, e.g., inflammatory and malignant cells, are active and determine the differentiation process. The pleiotropic plasticity of mononuclear phagocytes created through differentiation of blood MO is enormous and still not completely understood: this does not only pertain to the generation of heterogeneity among the various organ subpopulations of the MPS but also to the reactive and inflammatory cell types within this cell system. Hence, a small developing or metastasizing tumor cell aggregate may encounter totally different macrophages as effector cells of the body’s defense system than could established tumors where TAM macrophages may be something like the physiological organ-specific subtype, similar to K upfer cells in the liver and osteoclasts in the bone matrix. Thus, at least in some tumors or at certain stages of tumor progression, MO emigrating from the capillary bed are highjacked and re-educated to serve within the machinery of the growing tumor. Once established, the level of TAM macrophages may only partly depend on the influx of young MO while mainly relying on the self-renewal through in situ replication.

APPROACHES OF MACROPHAGE-BASED IMMUNOLOGICAL THERAPIES

The systemic activation of MAC through the use of lymphokines, synthetic analogs of muramyl dipeptide both as free and liposome-encapsulated drug had limited clinical effects so far [17, 18]; one reason being that this approach requires responsive but not symbiotic MAC at the tumor site.

Thus adoptive transfer of ex vivo-generated MAC where both their differentiation and functional activation have been completed away from the silencing micro-milieu of the tumor may be a rationalistic and more promising way to break this type of immune tolerance. More than 20 years ago Isaac J. Fidler and colleagues in Houston pioneered the use of activated MAC in murine models of metastatic tumor. Using the B16 melanoma model, they demonstrated that the percentage of animals cured and suppression of pulmonary metastasis depended on the in vitro activation as well as the number of MAC infused [19]. Other groups have confirmed their findings using different tumor models, including human xenografts [20–23]. It is, however, important to note that in these rodent studies all types of MAC-targeted immunotherapy was effective only in inhibiting metastasis formation, whereas regression of primary tumors was rarely observed [18]. This phenomenon might be explained by the size of the primary tumor but may also be due to the substantial differences in the functional competence of host MAC, which interact either with an established tumor or with small aggregates of metastasizing cells most often embedded in a cellular inflammatory reaction.

ADOPTIVE IMMUNOTHERAPY WITH HUMAN MAC CELLS

Although murine MAC can easily be obtained either from the peritoneal cavity or grown from bone marrow precursor cells, human studies on MAC adoptive transfer had to wait for appropriate technology to isolate or grow human MAC at large scale. Because blood MO are readily accessible and could be obtained in large quantities by leukapheresis and subsequent countercurrent centrifugal elutriation [24, 25], they were the first to be used in cancer patients [26, 27]. In fact up to $3 \times 10^9$ MO can be isolated through a single apheresis processing 8–10 L of blood. Yet, MO are still immature precursor cells that only on further differentiation acquire the full antineoplastic armamentarium [28–32] and then, when infused and having infiltrated into the tumor tissue, may be silenced along the same way as believed to take place in the ontogeny of TAM. Therefore the scheme of MAC adoptive therapy has to include terminal maturation of MO first, then followed by functional activation with interferon (IFN)-γ and reinfusion. Upon culture in the presence of autologous serum for 7 days MO indeed transform to mature MAC as evident by morphology and cytochemistry [28, 32, 33], analysis of maturation-dependent antigen expression [34, 35], cytokine repertoire [30], and development of both direct cell-mediated [32] as well as antibody-dependent cellular cytotoxicity [36]. Figure 1 demonstrates the functional changes occurring during differentiation. Grown from mononuclear cells on hydrophobic Teflon foils [28] to which they only loosely adhere, MO-derived MAC can easily be detached and recovered at day 7 and purified by elutriation from remaining lymphocytes [37, 38] before being retransfused to the patient. Figure 2 depicts the scheme of MAC adoptive immunotherapy. The recovery of MAC from blood MO seeded initially can be increased by the addition of granulocyte-macrophage colony-stimulating factor (GM-CSF) [39]. Serum-free conditions have also been developed [40–42].

First dose escalation studies starting with $10^5$ cells were initiated in 1988 first in Freiburg [43] and later in Strasbourg [44, 45]. It was demonstrated in these and subsequent studies [46–49] that in vitro-grown MAC could be safely administered at up to $3 \times 10^9$ cells per single infusion (maximum cell number achieved by leukapheresis) without major toxicity. Low-grade fever and flu-like symptoms were the only clinical side effects observed. MO activity could be followed by an increase in serum neopterin [43] and C-reactive protein as well as a transient cell-dose-dependent rise of thrombin-anti-thrombin (TAT) complexes [43, 48], reflecting the pro-coagulatory activity of IFN-γ-stimulated MO-derived MAC [50]. MAC therapies were repeated every 7–14 days, again without evidence of any cumulative toxicity. Similar dose escalation studies were performed reinfusing MAC in the peritoneal and pleural cavity and through regional liver perfusion via selective catheterization of the hepatic artery [51]. To increase the number of MO available for in vitro differentiation GM-CSF was administered for 7 days to patients before leukapheresis [49, 52]. This allowed an increase up to $7.3 \times 10^9$ MAC and to double the amount of cells given at a single infusion [52]. Apart from increasing the number of circulating MO it could be shown by whole-blood
assay that GM-CSF treatment induced functional changes in blood MO, e.g., an increase in tumor necrosis factor-α and a reduction in interleukin (IL)-10 secretion [B. Hennemann and R. Andreesen, unpublished results]. Furthermore, to also activate the cytotoxic cytokine repertoire of MAC, bacterial endotoxin [lipopolysaccharide (LPS), Salmonella typhimurium] was used for additional activation [53] in one study. Here, a maximal tolerated dose of $2 \times 10^8$ cells was established with chills, nausea, and headache being the limiting toxicities. However, no side effects were observed when patients were pretreated with ibuprofen, which allowed an increase in MAC numbers up to $1.5 \times 10^9$ per infusion without dose-limiting toxicity. In patients given LPS/IFN-γ-activated MAC a biological response could be monitored by analyzing serum levels of

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**Fig. 1.** Functional competence of human macrophages developing in vitro from cultured peripheral blood monocytes. Monocytes were cultured at $10^5$/mL RPMI 1640 plus 2% AB-group serum in hydrophobic Teflon bags for up to 11 days, harvested at indicated time points, and subjected to analysis for cytokine secretion, CD16 expression, and tumor cytotoxicity. (A) cell-mediated cytotoxicity against U937 cells on 18-h activation by 200 IU/mL IFN-γ measured in a post-labeling assay [29]; (B) CD16 expression as analyzed by immunocytology (left axis, ref. 35) and antibody-dependent cellular cytotoxicity against the cervical carcinoma line A431 using an anti-EGF-receptor antibody (right axis); (C) TNF-α [30] and MCT170 [31] concentration after 18-h activation with 100 ng/mL LPS abortus equi; (D) LPS-stimulated production of GM-CSF [82] and constitutive production of M-CSF [30] into 24-h supernatants of $10^5$ cells.
IL-6, IL-8, and IL-1 receptor antagonist increasing on infusion [53].

Labeling the autografted MAC with $^{111}$Indium-oxine revealed that intravenously reinfused cells were retained in the lungs for 45–90 min and then pooled into liver and spleen, concentrating at or around metastatic nodules [J. Marienhagen, B. Hennemann, R. Andreesen, unpublished results]. MAC injected intraperitoneally remained in the peritoneal cavity for more than 7 days and concentrated at sites of major tumor growth. Similar to the slow passage through the lungs, MAC infused via the hepatic artery were retained in the liver for about 30 min before being released into the venous circulation. Again, they seem to be localized at sites of metastasis where they could be detected for more than 7 days [51].

The clinical evidence of an antitumor effect of MAC adoptive therapy as of today is rather limited. The reduction of malignant ascites seen in three out of seven patients treated intraperitoneally might in fact be due to nonspecific inflammatory rather than antineoplastic effects because no change in tumor activity could be detected by computed tomography or laboratory testing [43]. Table 1 gives an overview of the current clinical studies performed using either MO and/or MO-derived MAC.

**TABLE 1.** Macrophage Adoptive Immunotherapy: Current Clinical Experience

<table>
<thead>
<tr>
<th>Center</th>
<th>Patients (n)</th>
<th>Cancer</th>
<th>In vitro stimulus</th>
<th>Route</th>
<th>Side effects</th>
<th>Clinical response</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bethesda a</td>
<td>7</td>
<td>Colorectal</td>
<td>IFN-γ</td>
<td>i.p.</td>
<td>Low-grade fever, peritoneal irritation</td>
<td>None</td>
<td>Stevenson et al. [26]</td>
</tr>
<tr>
<td>Freiburg</td>
<td>8</td>
<td>Various</td>
<td>IFN-γ</td>
<td>i.v.</td>
<td>Low-grade fever</td>
<td>None</td>
<td>Andreesen et al. [43]</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Ovarian, gastric</td>
<td>IFN-γ</td>
<td>i.p.</td>
<td>Low-grade fever, peritoneal discomfort</td>
<td>None</td>
<td>Andreesen et al. [43]</td>
</tr>
<tr>
<td>Strasbourg</td>
<td>10</td>
<td>Colorectal</td>
<td>IFN-γ</td>
<td>Hepatic artery</td>
<td>Flu-like symptoms</td>
<td>None</td>
<td>Hennemann et al. [51]</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Non-small-cell lung</td>
<td>IFN-γ</td>
<td>i.v.</td>
<td>Fever, dyspnea</td>
<td>None</td>
<td>Faradji et al. [44]</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Carcinomatosis</td>
<td>Liposomal MTP-PE</td>
<td>i.p. a</td>
<td>Fever, chills</td>
<td>1 PR</td>
<td>Faradji et al. [45]</td>
</tr>
<tr>
<td>Paris</td>
<td>12</td>
<td>Various</td>
<td>IFN-γ</td>
<td>i.v.</td>
<td>Fever</td>
<td>1 PR</td>
<td>Lopez [46]</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Mesothelioma</td>
<td>IFN-γ</td>
<td>Intrapleural</td>
<td>Fever, local pain</td>
<td>No data</td>
<td>Lopez, personal communication</td>
</tr>
<tr>
<td>Reims</td>
<td>15</td>
<td>Colorectal</td>
<td>IFN-γ</td>
<td>i.v.</td>
<td>Low-grade fever</td>
<td>None</td>
<td>Eymard et al. [47]</td>
</tr>
<tr>
<td>Rennes</td>
<td>15</td>
<td>Renal</td>
<td>IFN-γ</td>
<td>i.v. c</td>
<td>Nausea, low-grade fever</td>
<td>1 PR, 6 SD</td>
<td>Toujas, personal communication</td>
</tr>
<tr>
<td>Regensburg</td>
<td>9</td>
<td>Various</td>
<td>IFN-γ/LPS</td>
<td>i.v.</td>
<td>Fever, chills, hypotension</td>
<td>None</td>
<td>Hennemann et al. [53]</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Melanoma</td>
<td>IFN-γ</td>
<td>i.v. c</td>
<td>Low-grade fever</td>
<td>None</td>
<td>Hennemann et al. [52]</td>
</tr>
</tbody>
</table>

a Blood monocytes cultured overnight. b Reduction of malignant ascites in three of seven patients. c Pretreatment with 5 and 10 µg/kg GM-CSF for 7 and 14 days s.c.
FURTHER DEVELOPMENT AND PERSPECTIVES

The disappointing results from the current clinical protocols have prompted a second generation of trials, presently being designed and initiated. First, adoptive MAC therapy is being used in conjunction with multicyclic high-dose chemotherapy supported by peripheral stem cell transplantation to study the efficacy of MAC against low tumor burden. In this context possible antimicrobial effects and stimulation of hematopoiesis by MAC products will be assessed by comparing the incidence of febrile episodes and documented infections as well as the time to hematological reconstitution between two cycles of high-dose therapy, with one being supported by MAC autografting at day 3 after transplantation. Also, MAC adoptive therapy will be performed in leukemic patients in second complete remission with minimal residual disease being at a high risk of relapse.

Second, current protocols in patients with malignant glioblastoma and ovarian cancer combine adoptively transferred MAC with bi-specific antibodies directed against the FcγR1 (CD64) and either the epidermal growth factor receptor or the overexpressed HER2/neu protein, respectively [54, 55]. With these ongoing trials in Europe and the United States MAC are not only targeted to the tumor for intensification of their tumor cytotoxic reaction but are also aimed to induce a specific immune response through cellular and humoral mechanisms [56].

Third, MAC could be used for gene therapy of genetic disorders [57–59], to deliver hypoxia-regulated genes to tumor sites, or could be transfected to overexpress cytokines, augmenting their cytotoxic as well as their accessory function (Fig. 3).

MONOCYTE-DERIVED CELLS TO PRESENT ANTGEN

By changing the culture conditions MO can differentiate not only into phagocytic and cytotoxic MAC but under the influence of IL-4, GM-CSF, and TNF-α also to highly potent antigen-presenting cells (Fig. 4) exhibiting most if not all the features of professional antigen-presenting cells, the dendritic cells whose ontogeny have in fact recently been linked to the MPS [60–64]. These MO-derived dendritic cells (MO-DC) seem to be equivalent to DC derived from bone marrow progenitor cells [65] and have already been successfully used as cellular vaccines in clinical trials [66]; additional data is available from several animal trials. Pulsed in vitro with whole tumor-derived DNA [67], tumor-associated antigenic peptides [68, 69], crude tumor lysates [69, 70], or fused to generate tumor-DC hybrids [71], they are injected subcutaneously, intravenously, or intra-lymphatically to initiate or boost the patient’s specific immune response to the tumor (Fig. 5) [72, 73]. Dendritic cells might even be grown from leukemic monocytes to be used as vaccines in vivo for generating a cytotoxic T cell response directed against the leukemia-specific fusion protein resulting from the chromosomal translocation (e.g., CML) [74].

Yet, several questions remain to be answered: first, antigen presentation can induce both T cell activation, leading to induction of an immune response, and T cell anergy and tolerance induction [75, 76]. Thus, the specific type of DC being used for cellular therapy may be of crucial importance, especially as recent publications suggest an essential role of DC to apparently decide on an immunogenic or a tolerogenic response of the recognizing T cell [77]. In terms of function and phenotype, DC cultured from CD34+ hematopoietic precursors or MO, respectively, are quite similar [65], but little is known so

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**Fig. 3.** Perspectives in using monocyte-derived cells for adoptive transfer.

**Fig. 4.** Phenotypic and functional ability of monocyte-derived dendritic cells [65]. Monocytes were cultured in plastic flasks at 10⁵/mL RPMI 1640 with IL-4 (20 ng/mL), GM-CSF (10 ng/mL), and TNF-α (10 ng/mL) in the absence of serum. Freshly isolated MO and MO-derived DC on day 7 of culture were compared. (A) Stimulation of a primary 5-day mixed lymphocyte reaction (10⁵/well lymphocytes) using two different stimulator concentrations, overnight pulse with [³H]thy midine; (B) expression of CD1a and CD80 by flow cytometry.
far as to which cytokines can modulate DC biology in a way that optimizes the induction of anti-tumor immunity in vivo. A similar concern is expressed with respect to cell dose, optimal treatment schedule, as well as the route of application.

To take the concept of antigen-presenting cells a step further, it is tempting to also explore the possible exploitation of their ability to induce cytotoxic T cells specific for tumor-associated antigens in vitro [78, 79], which subsequently on expansion and activation can be used as the therapeutic cellular product (Fig. 5). Apart from immunotherapy of tumors other areas of medicine might benefit from DC technology: DC treated with IL-10 that down-modulates costimulatory molecules and suppresses mixed lymphocyte reaction may be able to generate tolerogenic T cells [80], which can be used in the setting of alllogenic bone marrow transplantation to reduce mortality from graft-versus-host disease. Also, donor-derived DC might help to reconstitute the long-lasting immunodeficiency after haplo-identical bone marrow transplantation.

CONCLUSIONS

Large quantities of highly competent tumor cytotoxic MAC can be generated in vitro from circulating blood MO and are well tolerated when reinfused into the autologous host. Although active in limiting the metastatic growth in murine tumor models, no significant clinical response has yet been documented in more than 100 patients treated at 7 different European centers. Ongoing trials will concentrate on the use of MAC autografts in combination with bi-specific antibodies following high-dose stem cell-supported chemotherapy and to combat minimal residual disease. It is suggested that future perspectives in the use of the cells of the MPS in adoptive immunotherapy have to be developed toward generating antigen-presenting DC derived from blood MO. These DC could be primed ex vivo with tumor-relevant antigens, either by incubation with tumor cell fragments, native or synthetic peptides, by transduction with genes encoding tumor antigens, or by fusing them with the autologous tumor cells. Primed DC can be directly infused as cellular vaccines or taken to induce and expand cytotoxic T cell products in vitro. DC or MAC might even be manipulated to induce tolerogenic rather than immuno-

genic T cells that could be used to reduce mortality from graft versus host disease after unrelated bone marrow transplantation.

REFERENCES


