Chapter 3

DIFFERENTIAL MODULATION OF MACROPHAGE ACTIVITY BY AK-5
3.1. INTRODUCTION

One of the many functions of Mφs in host immune response is their cytotoxic ability towards tumor cells [Ting et al., 1979; Ting and Rodrigues, 1979]. The variation in the levels of activation of Mφs towards different tumors depends on a host of factors, including the cytokine repertoire at the tumor origin [Bottazzi et al., 1983], the site of tumor growth [Garner et al., 1987], and the immune response elicited [Sotomayor et al., 1991].

The AK-5 tumor cells grow as a solid tumor and as ascites when transplanted s.c. and i.p., respectively [Khar, 1986]. The s.c.-transplanted tumor is highly immunogenic and regresses spontaneously by day 25-30 after injection; however, the i.p.-transplanted tumor grows rapidly and kills 100% hosts by day 6-10 [Khar, 1993]. Similar phenomena have been reported in the case of IL-2 gene-modified Lewis lung carcinoma cells (LLC-IL-2) in C57BL/6 mice [Heike et al., 1997], human endometrial carcinoma cell line in nude mice [Rubin et al., 1992], Theileria-infected bovine cell lines in SCID mice [Fell et al., 1990], ETN-1 human carcinoma in nude mice [Kruitwagen et al., 1989], and hamster pancreatic carcinoma in nude mice [Konishi et al., 1978]. In all cases, tumor cells inoculated s.c. produced well-differentiated adenomatous tumor nodules with the formation of glandular lumina and basal lamina. Tumor cells injected i.p. produced malignant ascites. Detailed study of the immune response in each of these cases was however, not elucidated.

Mφ activation is the acquisition of enhanced competence to complete a complex function; such as chemotaxis, destruction of microbes, lysis of tumor cells, phagocytosis, processing and presentation of antigen, regulation of hematopoiesis, or acceleration in the formation of an artherosclerotic plaque [Roitt and Delves, 1992]. Mφs are avidly phagocytic and also secrete or release a wide variety of toxic products, including acid hydrolases, neutral proteases,
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lipases, DNases, and reactive nitrogen and oxygen intermediates (RNI and ROI). RNI, ROI and TNF-α mediate MΦ activity during tumor growth [Roitt and Delves, 1992]. However, many tumors have the capacity to escape immune surveillance by a variety of methods [Szuro-Sudol and Nathan, 1982; Ting and Hargrove, 1982].

Earlier observations in our system revealed that in the s.c.-tumor transplanted animals, the peritoneal MΦs were highly activated [Bhaumik and Khar, 1998a]. On the other hand, in i.p. tumor-transplanted animals, the immune system remained suppressed and the peritoneal MΦs lost their tumoricidal function progressively and, by day 5, were no longer found in the peritoneum [Bhaumik and Khar, 1998b].

The mechanisms involved in the activation or suppression of the immune system by the tumor cells, which is also dependent on the site of tumor transplantation, have been studied. AK-5 tumor cells, when transplanted s.c., activated peritoneal MΦs through the induction of different cytokines, whereas tumor cells when transplanted i.p., suppressed the activity of MΦs, as well the production of these cytokines. Suppression of MΦ function was also observed when they were co-cultured with tumor cells in vitro. Our studies emphasize the capabilities of the AK-5 tumor in driving the immune response either towards an activated status or towards immune suppression, depending on the site of transplantation.

Preliminary studies have also been conducted on the appearance of the receptor NKG2D/NKR-P2 on activated peritoneal MΦs in the s.c.-injected animal. An important mode of immune recognition employed by NK cells is "induced self-recognition", exemplified by the NKG2D receptor-ligand system. The NKG2D immunoreceptor, expressed by NK cells, and by activated CD8+ T cells and MΦs, recognizes one of several cell surface ligands that are distantly related
to MHC class I molecules (i.e. H60 and Rae1 proteins in mice, and MHC class I chain-related proteins and UL-16-binding proteins in humans) [Diefenbach et al., 2000]. Cross-linking of NKG2D with its ligands has been found to activate NK cells and Mφs [Diefenbach et al., 2003].

Our studies indicate that Mφs are not specifically activated by AK-5 cells, but require other stimulants, such as cytokines, for the initiation of tumoricidal activity, and corroborates the earlier theory that the NK cell is the predominant activating cell type in our system.

3.2. RESULTS

3.2.1. FREE RADICAL PRODUCTION BY Mφs FROM TUMOR-BEARING HOSTS

3.2.1.1. Kinetics of the appearance of nitrite in the medium

The time-period required for the appearance of nitrite in the medium of cultured Mφs from naïve, as well as s.c. tumor-injected rats of different days was first checked. The results indicated that (i) the appearance of nitrite in the medium could optimally be detected after 18 h (Fig. 3.1A) and; (ii) Mφs from s.c.-injected rats of different days were progressively activated, with maximum activation at day 20, after which the NO secretion decreased to basal levels (Fig. 3.1B).
Fig. 3.1. Nitrite estimation in macrophage supernatants cultured *in vitro*, which had been isolated on different days from the peritoneum of rats injected s.c. with AK-5 cells. (A) Time-point study of the production of nitrite from Mφs isolated on days 0, 10, 20 and 30 after s.c. injection. Mφs were plated in IMDM and nitrite assay by Greiss reaction was conducted every 2 h; (B) Nitrite production of the same Mφs after 18 h, showing the difference in nitrite production, and hence the activation of peritoneal Mφs from s.c. tumor injected rats.
3.2.1.2. Kinetics study after co-culture of AK-5 cells and Mφs

Kinetics experiments were done to assess the effect of different ratios of AK-5 cells on Mφs. The viability of Mφs, as well as their NO secretory capacity as a function of their tumoricidal activity, were assessed (Fig. 3.2).

![Graph showing nitrite levels and Mφ viability over time](image)

Fig. 3.2. Nitrite levels (given as absorbance at 540 nm) in the supernatants after co-culture of Mφs with AK-5 cells at different time points. Co-culture with 1:3, 1:5 and 1:10 ratios of Mφs to AK-5 cells was done for different time-points, after which the AK-5 cells were washed off with PBS, and the Mφs were further incubated for 12 h. Control Mφs were also similarly treated. Open bars: nitrite levels, measured by Greiss reaction; solid bars: Mφ viability, determined by MTT assay. Mφ viability decreased significantly after 12 h co-culture with tumor cells, whereas nitrite levels decreased from 4 h onwards.

It was found that 1:5 ratio of Mφ to AK-5 cells for 4 h co-culture resulted in ~50% down-regulation with no loss in viability and hence, this was taken as the optimal ratio and time for co-culture in subsequent experiments. Low ratio of AK-
5 cells did not produce any activation (Fig. 3.3), but higher ratios caused down-regulation of NO production by the Mφs.

![Graph showing nitrite produced (μM) vs. mφ : AK-5 ratio](image)

**Fig. 3.3.** Co-culture of Mφs with low and high ratios of AK-5 cells. Cells were co-cultured for 18 h. Nitrite in the medium was taken as a measure of activation or inactivation/down-regulation of Mφ activity. Low ratios of Mφ to AK-5 cells did not show any activation, whereas high ratios resulted in down-regulation of nitrite production.

### 3.2.1.3. Specificity of interaction between AK-5 and Mφs

To check whether the inactivation of Mφs by AK-5 cells was specific, due to molecular interaction, and not due to a non-specific inactivation, such as medium depletion etc., Mφs were co-cultured under similar conditions with AK-5 cells and a number of other tumor cell lines. Co-culture of Mφs with the different tumor cells was done at 1:5 ratio of Mφ to tumor cell for 4 h, the tumor cells were removed by washing with PBS, and Mφs were cultured for an additional 16 h, after which culture supernatants were collected for nitrite analysis. As seen in Figure 3.4, only AK-5 cells induced down-regulation of Mφs under these conditions.
Fig. 3.4. Co-culture of Mφs with different tumor cell lines at 1:5 ratio of Mφ to tumor cell for 4 h, after which the tumor cells were washed off and the Mφs were further incubated for 12 h. Nitrite in the medium was taken as a measure of Mφ activity. Only AK-5 cells caused down-regulation of macrophage NO secretory function.

3.2.1.4. Generation of RNI and ROI by peritoneal Mφs

Peritoneal Mφs from normal, i.p and s.c. tumor-transplanted animals were evaluated for their production of free radicals, NO and O$_2^\cdot$. The control Mφs from naive animals produced a basal level of NO (5 ± 1.5 μmoles/10$^6$ cells) and O$_2^\cdot$ (30 ± 3.3 nmoles/mg protein), as shown in Figure 3.5A and B (bar 3), whereas Mφs from s.c. tumor-bearing rats produced enhanced levels of NO and O$_2^\cdot$ (bar 1). However, Mφs from i.p tumor injected animals showed suppression in free radical generation from the first day after tumor transplantation (bar 4). This observation was mimicked under in vitro conditions, where co-culture of Mφs with AK-5 cells caused comparative suppression in the levels of both NO and O$_2^\cdot$ (Figs. 3.5A and B, bar 2).
Fig. 3.5. Production of free radicals by peritoneal Mϕs after s.c. or i.p. transplantation of AK-5 tumor. Bar 1, Mϕs from s.c. tumor-bearing rats on day 15; Bar 2, Mϕs co-cultured with AK-5 cells *in vitro*; Bar 3, normal peritoneal Mϕs; Bar 4, Mϕs from i.p. tumor-transplanted rats on day 1. (A) Production of nitric oxide, measured as nitrite by Greiss reaction. (B) Production of superoxide, measured by cytochrome c colorimetric assay. (C) Cytotoxic activity of Mϕs after 16 h chromium release assay.
3.2.2. MΦ-MEDIATED CYTOLYSIS

Cytotoxic activity was suppressed in MΦs from i.p tumor-injected animals (Fig. 3.5C, bar 4) and in MΦs co-cultured in vitro with AK-5 cells (bar 2), as compared to the maximal cytolysis activity in MΦs from day 15 (s.c.) tumor-bearing animals (bar 1), and that in normal MΦs (bar 3). In order to establish the inhibitory effect of AK-5 tumor cells on MΦs, a low dose (5 x 10^5 AK-5 cells) was injected i.p., and the cytotoxic potential of the MΦs was evaluated. MΦ cytotoxicity, which showed an initial up-regulation, decreased steadily by day 4 and was negligible by day 6 (Fig. 3.6), indicating that AK-5 cells were able to successfully suppress the tumoricidal capacity of the MΦs before it could fully develop.

![Graph showing cytotoxicity over days](image-url)

**Fig. 3.6.** Cytotoxicity of peritoneal MΦs isolated from rats injected with a reduced number of AK-5 cells i.p. (5 x 10^5 cells/animal) on days 0, 1, 3, 4, 5 and 6. All animals died on day 6. The MΦs were cultured in vitro for 16 h with ^51^Cr-labeled BC-8 cells for cytotoxicity assay.
3.2.3. NITROTYROSINE ESTIMATION AS AN INDICATOR OF PEROXYNITRITE FORMATION

Western analysis of serum samples from s.c. tumor-injected animals collected on different days showed a progressive increase in nitrotyrosine content (Fig. 3.7). The presence of nitrotyrosine in the serum was clearly seen from day 13 (lane 5), which reached peak levels by day 14 (lane 6), and then decreased progressively (Fig. 3.7A). However, no nitrotyrosine presence was seen in serum from i.p. tumor-bearing animals, even on day 1 after i.p. tumor injection (lane 1), though normal serum samples (lane C) showed a faint signal (Fig. 3.7B). Serum from s.c.-injected rats on day 15 (positive control) showed a strong nitrotyrosine signal (lane 5).

3.2.4. iNOS EXPRESSION IN Mφs

The down-regulation of the inducible form of NOS present in Mφs was observed following co-culture of Mφs with AK-5 cells for different time periods in vitro. As seen in Figure 3.7D, the expression of iNOS started diminishing from the first hour of co-culture (lane 1), and was negligible after 3 h of co-culture (lane 3). Control Mφs were cultured for 5 h, to confirm that the down-regulation was not due to culture conditions (lane C).

3.2.5. CYTOKINE PRODUCTION BY Mφs

Cytokines, like TNF-α and IL-12, are produced by activated Mφs, which in turn cause auto-activation as well as activation of other immune cells. TNF-α production was measured indirectly by estimating cytotoxic activity of the culture supernatants against L929 cells, which are sensitive to TNF-α. As shown in
Fig. 3.7. Expression of nitrotyrosine in the sera of s.c. and i.p. tumor-transplanted animals. (A) lanes 1–9 represent sera collected on days 1, 2, 9, 10, 13, 14, 16, 17 and 20 of AK-5 s.c.-transplanted rats; (B) lane C is normal rat serum control; lane 1, day 1; lane 2, day 2; lane 3, day 3; lane 4, day 4 sera samples of AK-5 i.p.-injected rats. Lane 5 is the day 15 s.c. tumor-injected rat serum (positive control); (C) cell lysates of Mφs co-cultured with AK-5 cells for different time points, showing the down-regulation of iNOS expression. Lane C, control Mφs incubated for 5 h; Lane 1, Mφs co-cultured with AK-5 cells for 1 h; Lane 2, co-culture for 3 h and Lane 3, after 5 h co-culture.
Figure 3.8A, the level of TNF-α was minimal in MΦ supernatants from day 1 i.p. tumor-bearing animals, whereas it was significantly high in MΦs from day 15 s.c. tumor-bearing animals, as compared to normal MΦs. IL-12 levels were estimated by ELISA and were negligible on day 1 after i.p. tumor transplantation, whereas in s.c. tumor-bearing animals, it was significantly higher, as compared to the control (Fig. 3.8B).

![Graph A: Cytotoxicity](image)

![Graph B: IL-12 Secretion](image)

Fig. 3.8. The production of TNF-α and IL-12 by peritoneal MΦs. (A) Cytotoxicity of L929 cells by MΦ supernatants in the TNF-α bioassay. 1 unit of TNF-α induces 50% cell death; (B) IL-12 secretion by MΦs. 'C' represents naïve MΦs, 'A' is activated MΦs of s.c.-injected rats on day 15; and 'S' is MΦs from i.p.-injected rats on day 1.

### 3.2.6. PHAGOCYTOSIS FUNCTION

Phagocytosis by MΦs was compared amongst MΦs isolated on different days from s.c.-injected rats. The phagocytic capability of MΦs increased...
progressively with their other functions. As seen in Figure 3.9, a 3 h-phagocytosis assay with FITC-labeled particles demonstrated that the phagocytic function of Mφs increased and was maximum on day 14 after s.c. injection.

The phagocytosis experiment was also conducted with Mφs that had been co-cultured in vitro with AK-5 cells (Fig. 3.10). There was no difference in the phagocytic abilities of Mφs before and after the co-culture, indicating that the inhibitory effect of AK-5 cells was specific to certain functions of the Mφs only.

3.2.7. NKR-P2 RECEPTOR UP-REGULATION IN ACTIVATED Mφs

The expression of NKR-P2 receptor was seen to increase progressively in peritoneal Mφs in AK-5 s.c.-injected rats isolated on different days (Fig. 3.11). The expression was highest in Mφs of days 15 and 22 after s.c. injection.

3.2.8. ELUCIDATION OF NKR-P2 FUNCTION

NKR-P2 receptor has been implicated in the activation of NK cells, DCs and Mφs against tumors [Diefenbach et al., 2003]. Hence, the question was whether AK-5 cells could activate Mφs for tumoricidal activity via an NKR-P2 ligand on the AK-5 surface. For this purpose, several in vitro experiments were conducted in which Mφs were co-cultured with AK-5 cells in the presence of NKR-P2 antibody for blocking the receptor-ligand interaction, after which the tumoricidal activity of Mφs was checked. As seen in Figure 3.12A, there was no change in the tumoricidal activity of activated Mφs against labeled AK-5 cells, as measured by $^{51}$Cr-assay. Naïve Mφs were also incubated with α-NKR-P2 antibody to see whether the antibody could activate Mφs by binding to the NKR-P2 receptor on the Mφ surface. Mφ activity was measured by its NO secretory capacity (Fig. 3.12B), and was seen to be unaffected by the presence of the antibody. As antibodies are known to activate Mφs via their Fc receptor, isotype
Fig. 3.9. Phagocytosis assay of peritoneal Mφs isolated on different days from AK-5 s.c.-injected rats. The Mφs were plated on coverslips, incubated for 3 h with FITC-labeled zymosan particles, washed and then fixed for confocal analysis. The nuclei were stained with DAPI to show the presence and location of cells. (A) Transmission photo of naïve Mφs; (B) FITC-labeled particles alone; (C) Mφs stained with DAPI; (D) Mφs from naïve rats; Mφs from day 4 (E); day 11 (F); day 14 (G); day 18 (H) and day 25 (I), AK-5 injected animals
Fig. 3.10. Phagocytosis assay for Mφs before and after co-culture with AK-5 cells. (A) Control Mφs; (B) Mφs after co-culture with AK-5 cells. The tumor cells were washed off before the phagocytosis assay was performed. (C) Graphical analysis showing equal phagocytic potential of both sets of Mφs.
**Fig. 3.11.** Expression of NKRP-2 by Mφs isolated from the peritoneum of s.c. AK-5 injected rats on days 0, 8, 15 and 22. α-NKRP-2 antibody was used to probe the cell surface of paraformaldehyde-fixed Mφs, followed by staining with FITC-linked secondary antibody. DAPI (red) was used to stain the nuclei to indicate the presence of cells. The fluorescence was observed under confocal microscope.
antibody was also kept as a control. Overall, this data was consistent with our earlier observations that AK-5 cells in low ratio failed to activate MΦs (Fig.3.3).

![Graph A](image)

**Fig. 3.12.** α-NKR-P2 blocking experiments. (A) Activated MΦs were incubated with labeled AK-5 cells in a 16 h cytotoxicity assay, in the presence of α-NKR-P2 antibody. Isotype antibody was kept in a separate set of co-culture as a control. There was no difference in cytotoxic potential of the MΦs in presence of either antibody. (B) NO secretory capacity of the activated MΦs was not altered with increasing concentrations of either antibody.

3.2.9. NK CELL ACTIVATION OF MΦs

Since naive MΦs themselves failed to get stimulated after contact with AK-5 cells *in vitro*, experiments were conducted to find out the mechanism of MΦ
activation against AK-5 tumor in vivo. The assumption was made of the presence of an activating cell type, which secreted stimulatory cytokines that would cause Mφ activation. The major cytokine for Mφ tumoricidal up-regulation is IFN-γ, secreted by NK cells and T cells. Based on earlier studies from our lab that the NK cell was the predominant immune effector cell involved in the regression of AK-5 tumor [Khar, 1993], it was hypothesized that it may also be involved in Mφ activation. To test this theory, naïve Mφs were co-cultured with different ratios of activated NK cells isolated from the spleen of s.c.-injected rats. As seen in Figure 3.13, Mφs were progressively activated by NK cells over a period of time. Hence, it may be concluded that in vivo, NK cells are directly activated by the AK-5 tumor cells and secrete IFN-γ, which in turn activates Mφs.

![Graph](image)

**Fig. 3.13.** NK cell-Mφ coculture in vitro. Naïve Mφs were co-cultured with activated NK cells in 1:1, 1:3, and 1:5 ratios of Mφ to NK cell. After co-culture for different periods of time, the NK cells were washed off, fresh medium was added and the Mφs were incubated further for 12 h. Nitrite estimated in the culture supernatants was secreted by Mφs.

### 3.3. DISCUSSION

The AK-5 tumor cells follow a site-specific growth pattern in syngeneic hosts. When injected s.c., the cells have a prolonged growth phase of 15 days, forming a solid tumor mass, which undergoes regression, and the animal becomes normal [Khar, 1993]. Cells injected i.p. have a short cell doubling time, leading to
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massive cellular accumulation (∼100 x 10^6 cells), resulting in host mortality within 6-10 days [Khar, 1986]. This short time duration with heavy tumor burden impairs and inactivates the immune system, whereas the prolonged growth phase in s.c.-transplanted animals leads to immune activation and tumor regression.

Reports of a syngeneic leukemia cell line induced by Friend virus in C57BL/6 mice by Ting’s group [Ting et al., 1977] showed a similar site-specific cellular growth pattern, as in AK-5 tumor. As few as 1 x 10^3 leukemia cells, when injected i.p., induced progressive tumor growth and killed the host, whereas s.c. inoculation of 50 x 10^6 cells produced a transient tumor with subsequent regression. Their studies indicated that such suppression was due to the presence of suppressor T cells and suppressor Mφs from spleen, thymus and lymph nodes [Ting and Zhang, 1983]. Peritoneal Mφs produced positive regulation of T-cell-mediated cytotoxic response against tumor cells [Ting and Rodrigues, 1982].

In our system, though it was known that the predominant effector cell type is the NK cell [Khar, 1993], the peritoneal Mφs from s.c. tumor-bearing rats were also found to play a major role in tumor regression by secretion of immunostimulatory cytokines, as well as free radicals, like NO, O_2^- and ONOO^- (Figs. 3.5 & 3.7). The importance of Mφs in preventing tumor growth can be emphasized by the fact that inhibition of their function in i.p.-injected animals led to the successful establishment of ascites in the peritoneal cavity and overall immune suppression.

These Mφs did not seem to have any specific activating cell receptor against any ligand on the AK-5 cell, explaining why co-culture with low numbers of AK-5 cells did not trigger tumoricidal function (Fig. 3.3); this would also indicate that AK-5 cells could not activate Mφs via the NKR-P2 receptor, though this receptor was over-expressed in activated peritoneal Mφs (Fig. 3.10). Importantly, the enhancement of the receptor expression was seen to take place in peritoneal Mφs, which had no direct contact with the AK-5 tumor at the s.c. site.
Hence, the up-regulation of NKR-P2 was probably due to interaction with another cell type, or due to the presence of IFN-γ. This was substantiated by in vitro experiments with α-NKR-P2 antibody, which did not trigger Mφ activation or block Mφ cytotoxicity (Fig. 3.11). Hence, an alternative role for the presence of NKR-P2 in the peritoneal Mφs needs to be elucidated.

Co-culture of naïve Mφs with immune, activated NK cells increased the tumoricidal activity of Mφs, indicating that the NK cells stimulated Mφs in the AK-5 model for AK-5 cytolysis (Fig. 3.12). The pathway for activation is well known, as activated NK cells secrete high levels of IFN-γ, the major activating cytokine for Mφs. However, the possibility of other molecular interactions between NK cells and Mφs, or other cell types participating in the activation process, cannot be ruled out.

NO is the chief effector molecule released by Mφs after immunological activation, and is a well-known cytotoxic molecule for invading microorganisms and tumor cells [Moncada et al., 1991]. Activated Mφs in our system showed several-fold increase of NO production in s.c. tumor-bearing rats, whereas it was virtually undetectable by the first day of i.p. injection (Fig. 3.5). Hence, the tumor gained a dominant position over the host immune system within 24 h of i.p. injection, as far as Mφ cytotoxicity was concerned. Similar results were observed with O₂⁻ and ONOO⁻ production (Fig. 3.7). The interaction of NO with O₂⁻ is a very rapid reaction, forming the highly reactive species, ONOO⁻. It has been proposed that the major cytotoxic effects of NO are the result of peroxynitrite-induced lysis of cells. 3-nitrotyrosine has been stipulated to be a marker of ONOO⁻, as the molecule nitrosylates free and protein-associated tyrosine residues [Eiserich et al., 1996]. The detection of such nitrosylated proteins therefore, serves as a marker for the presence of ONOO⁻.
Cytokines released by Mϕs, like IL-12, are critical for effective anti-tumor immunity, as they induce Th1 cytokine production and anti-tumor cell-mediated responses; they stimulate the formation of NO and are instrumental in determining the success or failure of the host to resist tumor growth [Trinchieri, 1995]. Hence, it may be assumed that their effective inhibition is synonymous to the shutdown of all immune anti-tumor activity. Similarly, the tumoricidal effect of TNF-α is well documented [Hori et al., 1987]. It has been earlier reported that AK-5 s.c. tumor remission occurs by apoptosis and necrosis [Khar, 1993], where TNF-α plays an important role. The up-regulation of IL-12 and TNF-α in Mϕs from s.c.-injected animals, in comparison to that of normal animals, and their down-regulation in Mϕs from i.p.-injected rats indicated that the tumor induced an immune response in s.c.-injected animals, but escaped immune surveillance in i.p.tumor-injected animals (Fig. 3.8).

Hence, it is clear that s.c. transplantation of the AK-5 tumor cells provided sufficient time to the host immune cells to generate a variety of interleukins, which are involved in the activation of different immune cells, including Mϕs. On the other hand, i.p. injection of tumor cells provided AK-5 cells a direct contact with the resident immune cells, specifically peritoneal Mϕs, resulting in the suppression of Mϕ activity. This was also confirmed in in vitro co-culture experiments. These observations suggest that the tumor plays an important role in the modulation of the immune function, thereby driving it either towards activation or suppression, depending on the site of transplantation.