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NATURAL CELL-MEDIATED IMMUNITY TO MOUSE MAMMARY TUMOR VIRUS AND ITS RELEVANCE TO HOST IMMUNE DEFENCES*

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The mouse mammary tumor virus (MTV), first discovered by Bittner in 1936 [1], is a B-type virus of the Retraviridae family [10] capable of inducing tumors of the mammary gland. The most common way of infection by MTV is through the maternal milk [1]. The milk-transmitted virus is called exogenous MTV and causes a high incidence of early occurring mammary tumors in certain mouse strains (e.g. C3H, RIII, A and CBA mice). Other mouse strains present a low incidence of mammary tumors usually occurring very late in life and do not produce MTV in their milk (e.g. BALB/c and C57Bl/6 mice). Some low-cancer strains can be transformed into high-cancer strains by foster nursing on mothers with MTV-containing milk (e.g. BALB/cf C3H mice). However, other mouse strains are resistant to the infection [14]. Thus, the variation in mammary tumor incidence among several inbred mouse strain can be attributed to genetically controlled differences in susceptibility to the disease. This aspect has recently been reviewed by Hilgers and Bentvelzen [14]. High-cancer strains can be transformed into low-cancer strains by cesarean derivation and subsequent foster nursing on strains as the C57Bl/6 mice (e.g. C3Hf mice). However, C3Hf mice still possess an endogenous MTV integrated in their genome, as also shown for other mouse strains with a low incidence of mammary tumors [14]. Thus, with the exception of the GR mouse strain, which has a high incidence of mammary tumors due to the endogenous MTV [14], this virus variant is usually considered weekly oncogenic. In an attempt to avoid further complications with distinctions that are not of great relevance to the subject of this review, the terms MTV-infected and MTV-free will hereafter only refer to the exogenous milk-transmitted virus.

Other factors beyond the genetic background are thought to play a role in MTV oncogenesis. A hormonal influence has been demonstrated in tumor development of MTV-infected mice (reviewed in ref. 22). Furthermore, the immune system is believed to be involved in MTV oncogenesis, even though its exact role is still a matter of debate. Early studies (reviewed in ref. 2) with in vivo transplants of mammary tumors in virus-

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infected mice have reached the conclusion that neonatal infection with MTV conferred immunological tolerance to the virus itself. In fact, MTV-positive tumors can be transplanted more readily into MTV-infected mice than into syngeneic MTV-free hosts; furthermore, MTV-free mice can be immunized more easily than MTV-infected mice against MTV-positive tumors. However, the interaction of the immune system with MTV is not irrelevant in oncogenesis, since several investigators have shown that in vivo immunosuppression in MTV-infected mice by such treatments as neonatal thymectomy [13, 21, 29, 30, 40] or injections of antilymphocyte serum [18] or cortisone [32] reduced the incidence and delayed the appearance of primary mammary tumors. In an attempt to clarify the complex relationship between MTV and the immune system, several studies have been performed in the last decade with the aid of newly developed in vitro assays. Thus, humoral [15, 16] and cellular immunity [3, 5–7, 9, 11, 19, 24, 35, 36, 39] to MTV could be shown in virus-infected mice.

The aim of this review is to summarize the results obtained by investigating the cell mediated immune response to MTV before the mammary tumor appearance and to discuss its relevance to host immune defence mechanisms.

NATURAL CELL-MEDIATED IMMUNITY TO MTV IN NEONATALLY INFECTED MICE

The first indication that MTV-infected mice have natural cellular immunity to MTV antigens was provided by Müller and Zotter [24]. Employing the direct macrophage migration inhibition assay, an in vitro measurement of delayed hypersensitivity reactions [24], these investigators showed that MTV-infected CBA/Bln mice were capable of recognizing MTV antigens obtained from a mammary carcinoma. In fact, peritoneal cells from CBA/Bln mice incubated with MTV, released factors capable of inhibiting the migration of the macrophages present among the lympoid cells of the peritoneal exudate. In the same study, peritoneal cells from MTV-free XVII/Bln mice were not inhibited in their migration by MTV.

The presence of natural cell-mediated immune responses against MTV antigens in virus-infected mice was confirmed and extended by Blair and coworkers [3, 5–7, 19]. They conducted an extensive series of experiments using spleen cells from BALB/c (MTV-free) and BALB/cf C3H (MTV-infected) mice against mammary tumor cells in visual microcytotoxicity assays. Splenocytes from BALB/cf C3H female mice between the ages of 8 and 32 weeks significantly inhibited growth and survival of target tumor cells [7].

Also employing BALB/c and BALB/cf C3H mice, raised in a different colony, Creemers and Bentvelzen [9], found that MTV-infected mice have spleen cells which proliferate in vitro in response to MTV from RIII milk, whereas splenocytes from MTV-free mice do not respond. The cellular immunity of splenocytes from BALB/cf C3H mice against MTV was confirmed in this study, employing the leukocyte adherence inhibition assay. However, significant inhibition was also obtained when Rauscher leukemia virus was used in the assay [9]. A specific anti-MTV response was detected by these investigators only in

mammary tumor-bearing mice. Therefore, they concluded that their BALB/cf C3H mice did not possess antiviral cell-mediated immunity before the appearance of the tumor.

In vitro proliferation of spleen cells response to Tween/ether-disrupted MTV was also shown by Gillette and Lowery [11], who studied MTV-infected RIII mice. This cell-mediated reactivity was confirmed employing indirect migration inhibition assays [11].

The indirect macrophage migration inhibition assay was also chosen by us [35, 36, 39] to investigate the reactivity to MTV of C3H/HeN mice, either infected with milk-transmitted exogenous MTV (C3H) or freed of the virus by cesarean derivation and foster nursing on C57Bl/6 mice (C3Hf). We found that spleen cells from the majority of individually tested C3H mice responded to MTV antigens by MIF production. This reactivity appeared at 14 weeks of age in C3H mice, but was no longer detectable at 36 weeks. In contrast, reactivity was never displayed by spleen cells of C3Hf mice. Splenocytes from C3H mice aged 14–20 weeks also produced a limited but significant amount of 'spontaneous' MIF upon in vitro incubation [35, 39]. This was probably due to the in vitro activation by the virus. In parallel to these specific antiviral responses, 14–20 week old C3H mice had elevated spontaneous macrophage cytolytic activity against tumor cells [38], whereas there was no difference in the natural killer (NK) activity of spleen cells from 6–38 week old C3H and C3Hf mice [35]. This last result was recently confirmed by Blair et al. [8], who employed also mice of other genotypes.

Interestingly, employing C3H and C3Hf mice in a radioactive cytotoxic test, Stutman [34] found that only 20% of the C3H mice younger than 90 days had cytotoxic lymph node cells against mammary tumors. This percentage increased to 40% in mice older than 90 days. C3Hf mice (generation F 74–75 since foster nursing) gave consistently negative results in the cytotoxic assay.

CELL-MEDIATED IMMUNITY TO MTV IN MICE HORIZONTALLY INFECTED BY THE VIRUS

The transmission of MTV from cage to cage or from MTV-infected to a MTV-free mice within the same cage is still a very controversial issue [14]. Among other results, immunological evidence has been provided supporting to the hypothesis of horizontal transmission of MTV [3, 5–7, 14, 19, 36]. Even though the results summarized below do not prove this hypothesis, it is of interest to discuss them, since apparent contradictions in reports about cell-mediated immunity against MTV could be explained by the existence of immune reactions activated by horizontally transmitted MTV.

In their in vitro cytotoxic assays against mammary tumors, Blair and coworkers [3, 5–7, 19] found that in MTV-free BALB/c mice older than 14 weeks, spleen cells were also specifically reactive against MTV-induced mammary tumor cells. This unexpected finding was explained by Blair and Lane [5] as horizontal transmission of MTV, since BALB/c mice raised in isolation were not reactive against MTV. We confirmed this result in a recent study where the natural cell-mediated immunity of horizontally infected BALB/c mice was assessed by the production of macrophage migration inhibitory factor (MIF) in response to MTV purified from cell cultures [36]. The cellular response to MTV of BALB/c mice is qualitatively different from that of BALB/cf C3H mice, since the

former cannot be blocked by pretreatment of target tumor cells with serum from BALB/cf C3H mice, whereas the latter can [5, 6]. Moreover, cytotoxic activity of spleen cells from BALB/c mice older than 14 weeks is not dependent on the presence of T cells as in BALB/cf C3H mice [3, 19]. These qualitative differences might be of in vivo relevance. In fact, Moore and Holben [23] found no increase in mammary tumors in MTV-free BALB/c and C57B1/6 mice caged with multiparous MTV-infected mice, even though those mice had a significantly elevated incidence of MTV antigen in the third lactation milk.

In contrast with these results, Lopez and Sigel's group [20, 31] reported that female BALB/c mice were reactive to MTV antigens, whereas BALB/cf mice failed to respond. These investigators assessed cell-mediated reactivities by the direct migration inhibition assay and lymphocyte proliferation in response to milk-derived MTV. Since the BALB/c mice used in these studies were kept carefully in separate animal facilities from the MTV-infected BALB/cf C3H mice, Sigel et al. [31] tend to rule out the hypothesis of horizontal transmission. They suggest that the virus-associated antigens expressed in their BALB/c colony represent the activation of an endogenous gene coding for a MTV protein, whereas the lack of immunity of BALB/cf C3H mice is due to tolerance to MTV antigens [20, 31].

Finally, in the above-mentioned study by Gillette and Lowery [11], MTV-free Swiss mice also proved reactive to MTV antigens, but the lack of appropriate controls makes it difficult to interpret this result as horizontal transmission of the immunity.

In conclusion, an increasing number of in vitro studies (summarized in Table 1) suggest that natural cell-mediated immunity against MTV antigens is generally expressed by MTV-infected mice. However, there are still some contradictory results. Several factors can be proposed to explain the discrepancies, such as differences in genotype, age and tumor incidence of the mice tested. In addition, part of the contradictions derives from the fact that cell-mediated immunity to MTV has been investigated as an isolated issue, without any attention being paid to its relevance to other host immune reactivities. This question will undoubtedly be greatly clarified by a better understanding of the interactions between antiviral responses and the whole immune system. For instance, the presence of feedback suppressor mechanisms activated by the viral infection could account for the lack of immunity to MTV described in certain conditions [9, 20, 31]. Studies concerning this hypothesis are considered in the next section.

INTERACTION BETWEEN ANTI-MTV CELLULAR IMMUNITY AND OTHER IMMUNE FUNCTIONS

In 1964, Martinez was the first to show that neonatal thymectomy reduces the incidence and increases the latency period of MTV-induced mammary tumors in C3H/Bi mice [21]. This observation was subsequently confirmed and extended. Yunis et al. [40] have shown that neonatally thymectomized MTV-positive C3H mice which received thymus grafts or spleen cells had an incidence of mammary tumors comparable to intact

TABLE 1

Natural cell-mediated immunity to MTV antigens

Authors	Assay system	Mouse strain	Result	References
Blair and Lane	a)Visual microcytoxicity	BALB/cfC3H[a]	Immune	3, 5–7, 19
		BALB/c	Non-immune	
Creemers and Bentvelzen	a) Lymphocyte proliferation	BALB/cfC3H[a]	Immune (non-specific)	9
	b) Lymphocyte adherence inhibition	BALB/c	Non-immune	
Gillette and Lowery	a) Macrophage migration inhibition	C3H[a]	Immune	11
		RIII[a]	Immune	
	b) Lymphocyte proliferation	NIH Swiss	Immune (?)	
Lopez and Siegel	a) Lymphocyte proliferation	BALB/cfC3H[a]	Non-immune	20, 31
	b) Macrophage migration inhibition	BALB/c	Immune (> 14 weeks)	
Müller and Zotter	a) Macrophage migration inhibition	CBA[a]	Immune	24
		XVII	Non-immune	
Stutman	a) Radioactive microtoxicity	C3H[a]	Immune (40% > 14 weeks)	34
		C3Hf	Non-immune	
Tagliabue and McCoy	a) Macrophage migration inhibition	C3H[a]	Immune (> 14 weeks)	35, 36, 39
		C3Hf	Non-immune	

[a] MTV-infected mouse strain.

C3H mice. Heppner et al. [13] have reported that neonatal thymectomy significantly reduced the number of mice which developed hyperplastic alveolar nodules after treatment with noduligenic hormones. Roubinian and Blair have reported that the incidence of mammary tumors in MTV-infected mice could be reduced only by the incomplete T-cell depletion resulting from partial neonatal thymectomy [29]. In a more recent study, Roubinian et al. have shown that incomplete T-cell depletion increased cell-mediated responses to MTV, whereas complete T-cell depletion totally eliminated the immune responses to MTV without affecting tumor incidence [30].

In support of the hypothesis that immunosuppression modifies MTV oncogenesis, Squartini and Bolis [33] have shown that splenectomy reduces the incidence but accelerates the appearance of mammary tumors in BALB/cf C3H. Moreover, immunosuppression with antilymphocyte serum [18] or cortisone [32] reduces the incidence of mammary tumors.

Based largely on these observations, Prehn and Lappé developed the theory of immunostimulation of tumor growth, suggesting that 'a little immunity may be beneficial to tumor growth' [28]. It can be suggested that the antiviral immunity could activate suppressor cells capable in turn of inducing a depression of the surveillance mechanisms against tumor cells. For instance, immunosuppressive treatments such as thymectomy may result in the paradoxical reduction of the tumor incidence by eliminating those T-cell

subsets responsible for the induction of suppressor cells. Indeed, evidence of a depressive role of MTV on immune functions was provided by Blair et al. [4], who reported that BALB/cf C3H female mice had lower hemagglutinating titers to sheep red blood cells and rejected skin allografts more slowly than syngeneic BALB/c mice. Griswold et al. [12] later described MTV-infectd C3HeB/DeJ mice with a reduced hypersensitivity response to methylated serum albumin. Finally, Oth and Sabolovic [26] have shown that (Swiss × C3H)F_1 male mice aged 16–20 weeks had a reduced capacity to produce plaque-forming cells in response to sheep red blood cells when foster-nursed on MTV-infected C3H mice.

Employing the C3H/C3Hf mouse system described above we found that the proliferative response to PHA, Con A and LPS of spleen cells of 14–20 week old C3H mice was significantly depressed in comparison with C3Hf mice of the same age [35]. This depression was associated to the appearance of suppressor macrophages [35].

Based on these observations the following hypothesis was formulated. As a result of neonatal infection with MTV, C3H mice at about 14 weeks of age develop cell-mediated immunity against viral antigens. This can be measured as lymphokine production either in vitro or in vivo. The interaction between lymphokines (macrophage migration inhibitory factor and macrophage activating factor are thought to be identical [27]) and macrophages results in activation of this cell subset. Thus, macrophages with an increased cytolytic activity can be observed in 14–20 week old C3H mice. As a further consequence of macrophage activation, increased suppressor activity develops. It has been shown previously that many of the stimuli leading to increased macrophage cytoxicity also induce suppressor activity [17]. These suppressor cells could in turn account for the depressed lymphoproliferative responses to mitogens by spleen cells from C3H mice. Thus, it is suggested that the induction of suppressor macrophages could serve as a mechanism employed by MTV to overcome the host immune system.

The in vivo relevance of these in vitro observations was assessed. We found a marked reduction in the plaque-forming cell number in 14–20 week old C3H mice immunized against sheep red blood cells [37]. Furthermore, C3H mice aged 14–20 weeks had shorter latency periods and survival times than younger C3H mice when transplanted with mammary tumors [37]. This was in net contrast with the antitumor response of the virus-free C3Hf mice, aged 14–20 weeks, which were more resistant to transplanted mammary tumors than younger C3Hf mice.

Taken together these results could indicate that MTV infection reduces the host's immune defences via suppressor cells activated by antiviral immune responses. The suppressor mechanism we described is just a portion of the complex array of reactions and interactions taking place in vivo in MTV-infected mice.

In vitro assays have provided the first step towards a fuller comprehension of the role of the immune system in MTV oncogenesis. The hypotheses formulated from these results will indicate the direction to be taken by future research. Much is still needed to clarify adequately the biology of MTV that in the last few years has often been associated with human breast cancer [24].

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