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CELL-MEDIATED IMMUNE RESPONSES AGAINST MAREK'S DISEASE VIRUS: RECENT DEVELOPMENT

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SUMMARY

Marek's disease virus (MDV) infection has been controlled effectively by vaccination. Humoral immune responses (neutralizing antibodies) and cell-mediated immune (CMI) responses [T cells, natural killer (NK) cells and macrophages] are involved in vaccine-induced protection. *In vivo* studies suggest that CMI responses, particularly T cell responses, play pivotal roles in protection against MDV infection. However, there are no *in vitro* system to detect and analyse MDV-specific T cells in chickens inoculated with oncogenic or non-oncogenic MDV vaccine strains. This lack of knowledge is caused by a combination of the absence of major histocompatibility complex (MHC)-defined cells that are susceptible to infection with MDV and the inability to infect target cells at a high multiplicity of infection due to the strictly cell-associated nature of MDV. Using MHC-defined reticuloendotheliosis virus (REV) transformed cell lines stably expressing MDV gene as target cells in chromium release assays, MDV-specific CD8+ cytotoxic T lymphocyte (CTL) responses can be demonstrated in chickens inoculated with MDV. Similar to herpes simplex virus-specific CTL responses, MDV-specific CTL responses might play pivotal roles in inducing protection.

Keywords: Marek's disease virus, cytotoxic T lymphocytes, reticuloendotheliosis virus.

INTRODUCTION

Marek's disease (MD) is an economically important lymphoproliferative disease of chickens caused by an oncogenic alphaherpesvirus; the Marek's disease virus (MDV) (reviewed by Calnek and Witter, 1991). MDV can be divided into three serotypes; MDV-1 consists of all oncogenic and attenuated strains (e.g., RB1B, GA5, JMI6), MDV-2 consists of non-oncogenic strains (e.g., SB-1) and MDV-3 consists of non-pathogenic strains from turkeys, also known as herpesvirus of turkey (HVT) (reviewed by Schat, 1985). Most of the naturally occurring tumours are CD4+/CD8- T cells (Schat *et al.,* 1991). However, CD4-/CD8+, CD4-/CD8- and CD4+/CD8+ cells can also be transformed experimentally (Schat *et al.,* 1991).

MDV was initially classified as a gammaherpesvirus based on its biological properties (reviewed by Roizman, 1990). However, the overall genome structures of MDV and HVT are closely related to alphaherpesviruses [e.g., herpes simplex virus (HSV)] (Buckmaster *et al.,* 1988). The construction of a *Bam*HI restriction enzyme map from MDV-1 DNA has facilitated the characterisation of MDV genes (Fukuchi *et al.,* 1984). Since then, MDV genes with homology to HSV genes encoding for immediate early (IE), early (E) and late (L) proteins and genes unique to MDV have been cloned and sequenced. MDV IE genes (ICP4, ICP22, ICP27), L genes (gB, gD, gE, gH, gI, gK, gL) and genes unique to MDV [pp38, pp24, ppl4, meq, A41, L1, several small cDNAs of the *Bam*HI-H family (open reading frames A, B, C, D, E and F)] have been cloned and sequenced (reviewed by Venugopal and Payne, 1995; Zelnik, 1995; Omar, 1997). Even though rapid progress has been made in cloning and characterising MDV genes, only limited information is available on their role in inducing cell-mediated immune (CMI) responses. In this review, the use of REV transformed cell lines in studying CMI responses against MDV antigens and the importance of CMI responses, particularly cytotoxic T lymphocyte (CTL) responses, in MDV vaccine-induced immunity and genetic resistance will be discussed.

CYTOTOXIC T LYMPHOCYTE RESPONSES AGAINST MAMMALIAN HERPESVIRUSES

In contrast to MDV infection, the importance of CTLs against murine HSV infections has been well documented. CTL responses against the L genes (gB, gC, gD) and IE genes (ICP4, ICP27) of HSV have been studied in some detail (reviewed by Schmid and Rouse, 1992). The recognition of specific proteins of herpesvirus, e.g., IE or L proteins by CTLs depends on the major histocompatibility complex (MHC)

antigens expressed. For example, during HSV infection, ICP27 and ICP4 are target antigens for CTLs in mice of H-2^d and H-2^k haplotypes, respectively (Martin *et al.*, 1990; Banks *et al.*, 1991) while gB is a target antigen for CTLs in mice of H-2^b and H-2^d haplotypes (Witmer *et al.*, 1990). CD4+ T cells and/or CD8+ CTLs against the HSV gB, gC, gD and ICP27 have been implicated in the protection against HSV challenge (Ghiasi *et al.*, 1994; Manickan, *et al.*, 1995).

IMMUNE RESPONSES AGAINST MAREK'S DISEASE VIRUS INFECTION

Introduction

It has been known for more than 20 years that chicks vaccinated at one day of age with one or more of MDV serotypes are protected against the disease. In addition, chickens vaccinated with bivalent or trivalent vaccines are better protected against MD than chickens vaccinated with monovalent serotype 2 or 3 vaccines (reviewed by Witter, 1985). Chickens selected to be genetically resistant to MD (MHC: B^{21} B^{21}) are better protected than susceptible chickens (MHC: B^{19} B^{19}) following vaccination with one or more serotypes of MDV (reviewed by Calnek, 1985). However, neither the protective antigen for the three serotypes of MDV nor the mechanisms of genetic resistance have been defined. It has been suggested that humoral and CMI responses are involved in vaccine-induced immunity (reviewed by Schat, 1996).

Humoral immune responses

Neutralizing antibodies (maternally derived or actively acquired) are important in modulating the early stage of MDV infection (reviewed by Powell, 1985). However, the role of different MDV antigens in inducing protective neutralizing antibodies has been poorly studied. Earlier studies reported that chickens infected with MDV develop antibodies against 35 different virus-specific proteins of which 18 appeared to be glycoproteins (van Zaane *et al.*, 1982). The importance of MDV gC and gB in inducing neutralizing antibodies has been examined by several investigators. Antibodies directed against gB (Ikuta *et al.*, 1984) but not gC (Kaaden, 1977) play an essential role in inducing protective immunity. Futhermore, Nazerian *et al.* (1992, 1996) reported that chickens of the B^2B^{15} haplotype infected with recombinant fowlpox virus (rFPV) expressing MDV gB (rFPV-gB) but not with rFPV expressing MDV pp38, gC, gD or tegument proteins were protected against MDV challenge. These chickens produced neutralizing antibodies but the possible role of CTL responses was not investigated.

T cell-mediated immune responses

Although *in vivo* studies using thymectomized chickens suggested that T cell responses play a dominant role in protection against challenge with MDV or MD tumour transplants (Gupta *et al.*, 1982), the study of CMI responses during MDV infection has been hampered due to at least two reasons. First, the highly cell-associated nature of MDV prevents infection of cells at a high multiplicity. Thus, the use of MDV-infected chicken kidney cells (CKC) as target cells in a 4-hr chromium release assay (CRA) was not very successful (Schat and Heller, 1985). Moreover, chicken embryo fibroblasts (CEF) cannot be used in this assay due to the low level of class 1 MHC expression (Dunon *et al.*, 1990).

The second problem is the lack of MHC-defined target cells that are susceptible to infection with MDV. The MDV tumour cell lines have been used as target cells in CRAs. Numerous investigators reported that spleen cells obtained from chickens inoculated with MDV-1 (Calnek *et al.*, 1979), MDV-2 (Schat and Calnek, 1980) or HVT (Sharma *et al.*, 1978) lyse only allogeneic but not syngeneic tumour cells. Sharma (1977) suggested that the phenotype of the effector cells is T cells since depletion of T cells abolished the cytolytic activity. However, Schat *et al.* (1982) reported that the allogeneic responses are directed against alloantigens and not against MD tumour-associated surface antigens. Thus, it is very unlikely that the allogeneic T cell responses play major roles in protection against MDV infection.

Other facets of immune responses

Besides humoral and T cell responses, other components of the immune system can modulate MDV infection. Kodoma *et al.* (1979) and Ross (1980) showed that antibody dependent cellular cytotoxicity can be demonstrated using antiserum from MDV- or HVT-infected chickens, in conjunction with normal splenic or peripheral blood lymphocytes. The importance of macrophages during MDV infection has been studied by treatments that selectively activate and inhibit the function of macrophages (Haffer *et al.*, 1979). Studies have shown that natural killer (NK) cells play important roles in curtailing viral replication (Heller and Schat, 1987) and inhibiting tumour progression (Lam and Linna, 1979a, 1979b). Chickens infected with MDV and HVT produce interferons (IFNs) (Kaleta and Bankowski, 1972). However, the

importance of the chicken IFN-α and -β and IFN-γ in modulating MD infection is not known. Based on the current understanding on the importance of mammalian IFN-γ during viral infection (reviewed by Biron, 1994), it is possible that IFN-γ plays a key role in the activation of macrophages, NK cells and T lymphocytes during the early stages of MDV vaccination or infection.

RECENT ADVANCES IN THE STUDY OF CYTOTOXIC T LYMPHOCYTE RESPONSES AGAINST MAREK'S DISEASE VIRUS INFECTION

In contrast to MDV infection, syngeneic killing of virus transformed cells by virus-specific CTLs can be demonstrated in chickens infected with reticuloendotheliosis (REV) (Maccubbin and Schierman, 1986). In these studies, REV-specific CTLs recognized REV-transformed cells in a syngeneic but not allogeneic MHC-restricted manner. Subsequent studies by Lillehoj *et al.* (1988) and Merkle *et al.* (1992) indicated that these CTLs are CD4-CD8+, Ia+ (class II MHC+) and TCR$\alpha\beta$+. The use of molecular biology techniques has raised a few new findings on the importance of CTL responses during MDV infection. As previously mentioned, splenic CD8+ CTLs obtained from REV infected chickens lysed REV-transformed cells in a syngeneic but not allogeneic manner (Maccubbin and Schierman, 1986). Thus, these REV transformed cell lines can serve as target cells to study CMI responses against MDV if the cells can be infected with MDV or transfected with MDV genes. Pratt *et al.* (1992b) and Uni *et al.* (1994) generated MHC defined REV-transformed cell lines stably expressing MDV pp38 antigen. These cell lines were generated by cotransfection of REV cell lines latently infected with MDV (Pratt *et al.*, 1992a) or REV cell lines free of MDV (Uni *et al.*, 1994) with MDV DNA fragments and an eukaryotic expression vector. In these studies the pp38 expressing REV cell lines were lysed by syngeneic cytotoxic spleen cells, presumably CTLs obtained from chickens infected with one of the three serotypes of MDV. However, the importance of individual MDV antigens in inducing CTL responses could not be determined with these target cell lines since the transfected MDV fragments encoded for several MDV genes (Pratt *el. al.*, 1992a).

Recently, Omar and Schat (1995a, 1995b, 1996c) developed MHC-defined REV cell lines stably expressing individual MDV genes. A total of eight MDV genes, pp38, *meq*, ICP4 and ICP22 genes of MDV-1 strain GA and gB, A41, ORF A and L1 of MDV-1 strain RB1B was stably transfected into REV cell lines expressing B^{21} B^{21} and B^{19} B^{19} antigens (Omar and Schat, 1996c). The B^{21} B^{21} and B^{19} B^{19} REV cell lines were developed from REV infected chickens that are genetically resistant and susceptible to MD, respectively. As shown in Tables 1 and 2, regardless of the genetic susceptibility to MD, CD8+/TCR$\alpha\beta$1+ CTL responses against pp38 and gB can be detected in chickens inoculated with an MDV-2 vaccine strain SB-1 (Omar and Schat, 1996a, 1996b, 1997). Similar results were obtained with chickens inoculated with an oncogenic MDV-1 JM-16 strain. However, chickens with these MHC haplotypes did not induce CTL responses against A41, L1, ORF A or ICP22 following infection with JMI6 or SB-1 (Omar and Schat, 1996c). The ability of JMI6- or SB-1-infected chickens to recognise pp38, ICP4 and gB of GA and RB1B strains, respectively, indicate that there might be common CTL epitopes among these serotypes. Cross-reactive CTL clones recognising more than one serotypes have been demonstrated in HSV (Bonneau *et al.*, 1993) and dengue virus (Zivny *et al.*, 1995) infections. Thus, the ability of pp38, gB and ICP4 to induce cross-reactive CTL responses suggests that these antigens might play a central role in vaccine-induced immunity confer by bivalent vaccines consisting of non-oncogenic MDV-2 and -3 or trivalent vaccines consisting of all three serotypes. The inability of JMI6- or SB-1-sensitized B^{21} B^{21} and B^{19} B^{19} chickens to lyse syngeneic cell lines expressing A41, L1, ORF A or ICP22 probably indicate that these genes do not play a major role in inducing CMI responses. However, based on the current understanding of class 1 MHC-restricted CTL responses in mammalian (reviewed by Germain, 1994), these MDV antigens might play an important role in inducing CTL responses in chickens with different MHC haplotypes.

The ability to demonstrate CD8+/TCR$\alpha\beta$+ CTLs against ICP4 in MD resistant B^{21} B^{21} chickens but not in susceptible B^{19} B^{19} chickens is intriguing (Tables 1 and 2). It is attractive to postulate that the development of ICP4-specific CTLs in the B^{21} B^{21} chickens might induce a protective anti-viral response that curtails MDV replication. This hypothesis is supported by studies on the pathogenesis of MDV (reviewed by Calnek, 1985). In addition, numerous studies have suggested that vaccination induces anti-viral responses and genetically resistant chickens have lower level of MDV replication than the susceptible chickens following vaccination with one or more MDV serotypes (Powell, 1985).

Table 1. Virus-specific cytotoxic T lymphocytes from 5-week-old B^{21} B^{21} Marek's disease resistant chickens seven days post infection with SB-1

Exp.	effector cell treatment[a]	% specific release ± SEM[b] CU-205 (parent)	CU-362 (pp38+)	CU-368 (gB+)	CU-375 (ICP4+)
1	none	1.0 ± 0.2	14.9 ± 0.8^{c}	13.1 ± 1.9^{d}	8.8 ± 1.1^{f}
	CD8+ depleted	1.4 ± 1.5	5.4 ± 1.8**	5.7 ± 1.4**	3.9 ± 1.8
	CD4+ depleted	-1.1 ± 0.5	8.7 ± 0.2^{e*}	8.8 ± 1.8^{f}	6.7 ± 0.6^{g}
2	none	1.6 ± 1.5	13.1 ± 1.7^{d}	15.9 ± 0.3^{c}	9.8 ± 2.2^{f}
	CD8+ depleted	0.8 ± 1.8	5.5 ± 0.8**	4.7 ± 1.6***	1.1 ± 1.4**
	$TCR^{\alpha\beta}2$+ depleted	0.1 ± 1.4	10.8 ± 1.4^{e}	12.1 ± 0.9^{d}	9.6 ± 2.2^{f}
3	none	2.9 ± 2.1	11.1 ± 2.2^{e}	12.6 ± 1.5^{d}	8.2 ± 2.49^{g}
	$TCR^{\alpha\beta}1$+ depleted	0.4 ± 1.4	3.6 ± 2.3*	2.9 ± 2.0**	1.2 ± 1.8*
	$TCR^{\gamma\delta}$+ depleted	2.6 ± 1.6	11.8 ± 0.6^{d}	14.6 ± 1.1^{c}	10.7 ± 2.9^{f}

[a] 5 x 10^6 effector cells (E:T ratio of 100: 1) of total spleen effector cell populations or effector cells depleted for a T cell subset.
[b] The mean % SR from six SB-1 infected chickens ± SEM.
Student's t test $^{c}p<0.001$, $^{d}p<0.005$, $^{e}p<0.01$, $^{f}p<0.025$, $^{g}p<0.05$ compared to uninfected spleens and $^{***}p<0.005$, $^{**}p<0.01$, $^{*}p<0.05$ compared to total spleen effector cell populations.

Table 2. Virus-specific cytotoxic T lynphocytes from 5-week-old $B^{19}B^{19}$ Marek's disease susceptible chickens seven days post infection with SB-1

Exp.	effector cell treatment[a]	% specific release ± SEM[b] CU-91 (parent)	CU-364 (pp38+)	CU-371 (gB+)	CU-373 (ICP4+)
1	none	2.2 ± 1.1	13.7 ± 0.5^{c}	12.7 ± 0. 1^{e}	3.0 ± 1.1
	CD8+ depleted	1.8 ± 0.5	7.5 ± 1.1^{g*}	6.4 ± 1.5*	-
	CD4+ depleted	1.4 ± 6.2	9.8 ± 0.8^{e}	9.6 ± 1.0^{e}	-
2	none	2.6 ± 0.8	11.6 ± 0.8^{d}	11.8 ± 1.7^{d}	1.0 ± 0.5
	$TCR^{\gamma\delta}$+depleted	2.8 ± 1.2	11.4 ± 0.8^{d}	13.9 ± 0.2^{c}	-
3	none	-0.9 ± 1.6	12.8 ± 1.7^{d}	12.7 ± 2.1^{d}	1.5 ± 0.7
	$TCR^{\alpha\beta}1$ + depleted	1.6 ± 1.4	3.4 ± 3.5***	6.0 ± 2.2*	-
	$TCR^{\alpha\beta}2$+ depleted	3.2 ± 1.9	9.9 ± 0.5^{e}	10.8 ± 1.6^{e}	-

[a] 5 x 10^6 effector cells (E:T ratio of 100:1) of total spleen effector cell populations or effector cells depleted for a T cell subset.
[b] The mean % SR from six SB-1 infected chickens ± SEM
Student's t test $^{c}p<0.001$, $^{d}p<0.005$, $^{e}p<0.01$, $^{f}p<0.025$, $^{g}p<0.05$ compared to uninfected spleens and $^{**}p<0.025$, $^{*}p<0.05$ compared to total spleen effector cell populations.

FUTURE DEVELOPMENT

Similar to the B^{19} B^{19} chickens infected with MDV, Omar *et al.* (submitted for publication) and Schat *et al.* (1996) showed that B^{19} B^{19} chickens vaccinated with rFPV-gB induce CTL responses against MDV gB. However, preliminary studies using passive transfer of gB-immune spleen cells failed to demonstrate the protective effect of these cells (Omar, 1997). The interpretation of this negative results is difficult because the transfer studies were complicated by a number of problems such as the immunological status of the recipient chickens and the low frequency of antigen-specific CTLs. The importance of MDV gB in vaccine-induced immunity need to be addressed, since HSV gB plays a key role in inducing protective humoral and CMI responses (Ghiasi *et al.,* 1994). *In vitro* stimulation and propagation of gB-specific CTL clones using irradiated rFPV-gB-infected CKC as antigen presenting cells and a highly purified chicken IL-2-like cytokine are underway (Markowski and Schat, unpublished data). The development of *in vitro* propagated gB-specific CD4+ and CD8+ T cells and passive transfer of these cells into immunologically compromised chickens might determine the importance of gB-specific T cells during MDV infection. Likewise, the development of ICP4-specific CTL clones might determine the importance of this antigen in inducing vaccine-induced immunity and genetic resistance. However, the development of antigen-specific CTL clones requires the use of chicken IL-2 which has not been cloned or fully characterised.

The molecular characterisation of avian MHC genes suggested that the chicken MHC appears to be simpler and more compact than the mammalian MHC (reviewed by Plachy *et al.,* 1992). Recently, it has been suggested that the chicken MHC genes might have a direct influence on disease resistance and this phenomenon might be immunologically related (reviewed by Kaufman *et al.,* 1995). Thus, studies using epitope tagging, mutagenesis of the class 1 binding sites and elution of self peptides associated with various MHC class I binding sites have just begun (Fulton *et al.,* 1995; Kaufman *et al.,* 1995). Characterisation of these peptides, their binding sites and the identification of virus-specific CTL epitopes are essential for the construction of novel vaccines expressing more than one CTL epitopes.

CONCLUSION

The REV-transformed cell lines can be used to study CTL responses against MDV and possibly against other avian infectious diseases. Although humoral response is important for vaccine-induced immunity, it can be argued that this type of response might not be sufficient to induce protection since MDV is highly cell-associated and will establish latent infections in T cells. Vaccine-induced protection is probably associated with anti-viral CTL responses, where the balance between CMI responses and the development of latently infected and transformed T cells is a key factor for protection. The preferential recognition of MDV antigen by CTL responses obtained from chickens with different MHC haplotypes suggests that the avian MHC genes might play an important role in regulating CTL responses. The identification of MHC-restricted CTL response and the molecular characterisation of the class 1 binding sites will provide new insights in avian cellular immunology and virology.

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RINGKASAN

GERAKBALAS IMUN BERANTARAKAN SEL TERHADAP VIRUS PENYAKIT MAREK: PERKEMBANGAN TERKINI

Jangkitan penyakit Marek (MDV) telah terkawal secara berkesan melalui pemvaksinan. Gerak balas imun humoral (antibodi peneutralan) dan gerak balas imun berantarakan sel (CMI) [sel T, sel pemusnah semulajadi (NK) dan makrofaj] terlibat dalam perlindungan teraruh vaksin. Kajian in vitro *menyarankan gerak balas CMI, terutama sekali gerak balas sel T, memainkan peranan penting dalam perlindungan tehadap MDV. Bagaimanapun, tiada sistem* in vitro *wujud untuk mengesan dan menganalisis sel T khusus MDV dalam ayam yang diinokulat dengan strain vaksin MDV onkogen atau bukan onkogen. Ketiadaan pengetahuan ini disebabkan oleh gabungan tiadanya sel tertakrif kompleks histoserasian major (MHC) yang rentan terhadap jangkitan MDV, dan ketakupayaan untuk menjangkiti sel sasar pada kegandaan jangkitan tinggi disebabkan sifat terkait sel ketat MDV. Dengan menggunakan titisan sel terjelma virus retikuloendoteliosis teraktif MHC (REV) yang secara stabil menyatakan gen MDV pada sel sasar dalam asai pembebasan kromium, gerak balas sel T sitotoksik CD8+ (CTL) khusus MDV boleh ditunjukkan pada ayam yang diinokulatkan dengan MDV. Gerak balas CTL khusus virus herpes simpleks, seperti juga gerak balas CTL khusus MDV memain peranan penting dalam pengaruhan perlindungan.*