IMMUNE MECHANISMS IN MAREK'S DISEASE

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Abstract—Resistance to progressive tumor development in MD is either naturally inherited or can be induced by vaccination with apathogenic or attenuated MDV or with HVT. Studies on the effects of immunosuppression on resistance have shown that natural and vaccine induced resistance may be mediated through immune responses. Cell-mediated immune responses rather than humoral responses appear to be of principal importance. The antigen(s) against which protective cell-mediated immunity is elicited are not yet clearly delineated. Both virus-related and tumor antigens may be involved. Progress in the understanding of cell-mediated immunity in MD has been slow because of lack of reproducible in vitro tests to measure this response in infected chickens. The development of lymphoblastoid cell lines from MD lymphomas, however, has enabled the development of an in vitro cytotoxicity test. In this test, which utilizes MSB-1 cells as the target cells, a specific cell-mediated immune response, presumably against the tumor antigen, MATSA, was detected in chickens infected with MDV. Further studies using similar in vitro tests will facilitate a better understanding of the role cell-mediated immune responses might play in development of MD.

Key words: Marek's disease, immunity, mechanisms, resistance

MÉCANISMES D'IMMUNITÉ DANS LA MALADIE DE MAREK

Résumé—La résistance au développement progressif de la tumeur dans le Marek's disease (M.D.) peut être soit naturellement héritée soit provoquée par la vaccination avec MDV apathogène ou atténué ou avec H.V.T. Les études sur les effets de l'immunosuppression ou de la résistance ont démontré que la résistance naturelle et celle provoquée par le vaccin peuvent être médiasées par les réponses immunisantes. Les réponses immunisantes médiasées par la cellule plutôt que les réponses humorales paraissent être d'une importance capitale. Le (s) antigène(s) contre lesquels l'immunité protectrice médiasée par la cellule est suscitée n'ont pas encore été délimités avec précision. Les antigènes de la tumeur et ceux apparentés au virus pourraient être impliqués. Le progrès face à la compréhension de l'immunité médiasée par la cellule a été lent à cause d'un manque d'épreuves in vitro reproductibles capables de mesurer cette réponse dans les poulets contaminés. Le développement de lignes de cellule lymphoblastoïdes des lymphomas M.D. cependant, a permis le développement d'une épreuve in vitro de cytotoxicité. Dans cette épreuve, qui utilise les cellules M.S.B.-1 comme cellules cibles, une réponse spécifique immunisante médiasée par la cellule, vraisemblablement contre l'antigène de la tumeur MATSA, a été décelée dans les poulets contaminés de M.D.V. D'autres études qui utiliseront des épreuves in vitro de la sorte faciliteront une meilleure compréhension du rôle les réponses immunisantes médiasées par la cellule pourraient jouer dans le développement du M.D.

Mots-clés: Maladie de Marek, immunité, mécanismes, résistance

INTRODUCTION

Marek's disease (MD) of chickens is a naturally occurring malignant lymphoid neoplasm caused by a cell-associated herpesvirus. The virus transforms thymus derived (T) lymphocytes [17, 19, 23, 24, 41], although the lymphomas that develop as a result of MDV infection, consist of usually a minor proportion of transformed cells together with a large number of apparently normal lymphoid cells of both B and T types [13, 30].
Although infection with MDV in chickens is immunosuppressive and impairs the ability of the host to respond to certain antigens both in the B and the T cells systems, the infected chickens mount a vigorous immune response to MDV and its antigens [20]. The understanding of these immune responses is of particular interest because of the possible role they might play in resistance to MD.

In this report we will briefly present models of resistance to MD and discuss the immune responses that are implicated in mechanisms of resistance.

RESISTANCE TO MD

A unique feature of MD is that this is the only known example of a naturally occurring malignant disease that can be prevented by vaccination. Of interest also is the phenomenon of natural resistance to the disease that is expressed by certain genetic lines independent of prior vaccination. Some of the important characteristics of resistance are briefly discussed below.

Vaccine induced resistance

Shortly after the discovery of MDV, several highly effective vaccines were developed that prevented mortality from MD. In general, three kinds of vaccines have been used; vaccines consisting of: (a) attenuated MDV [8], (b) naturally apathogenic MDV [29] and (c) another herpesvirus, namely, herpesvirus of turkeys (HVT) which is antigenically related to MDV [21]. Parameters of vaccination with all 3 types of vaccines are more or less similar [26], although HVT vaccine has been most commonly used in the field and will be considered further. Turkey is the only known natural host for HVT. Upon inoculation, chickens develop a persistant viremia but horizontal transmission of the virus among chickens does not generally occur [21, 27] or occurs to a limited extent [6]. The virus is largely non-pathogenic for both turkeys and chickens, although recently it was found [44] that HVT infection in chickens induced mild microscopic lymphoproliferation in nerves and gonads during early stages of infection. These mild lesions, however, disappear within about a week of development.

Chickens vaccinated with HVT show marked resistance to tumor formation by virulent MDV. The resistance in vaccinated chickens is to tumor formation and not to infection with MDV. Thus, MDV enters and replicates in cells of vaccinated chickens and even causes mild lesions [44] but simply fails to induce progressive tumors.

Natural resistance

Chickens of certain genetic lines are highly resistant to MD. This natural resistance is either expressed at hatching (early resistance) [9] or is developed gradually with age (late resistance) [32]. Presence of maternal antibody may enhance resistance [4] although resistance is expressed in the absence of this antibody [35]. Recent studies have shown that both early and late natural resistance is expressed through lesion regression [35, 37, 43]. Subsequent to infection with MDV, resistant chickens develop microscopic and sometimes gross lesions of MD which eventually disappear, whereas, lesion development in simultaneously infected susceptible chickens is accompanied by high levels of mortality.
The mechanisms by which either natural or vaccine induced resistance are mediated is not entirely clear, however, because in some resistant chickens lesions of MD develop and then are overcome it appeared that this resistance may be immunologic in character. Evidence for involvement of immune systems in expression of resistance in MD has been largely derived from studies with selective immunosuppression.

**EFFECT OF IMMUNOSUPPRESSION ON RESISTANCE**

It was observed earlier that chickens resistance to MD at hatching may have a selective ability to produce high levels of virus neutralising antibody implying that resistance may be dependent upon this selective antibody and that this ability may be genetically controlled [3, 36]. Later it became evident that the virus neutralising antibody was also produced by some susceptible chickens [33, 42]. The observation that agammaglobulinemic chickens were fully resistant to MD [34] firmly established that resistance was not solely mediated through humoral immunity. B cell suppression induced by neonatal treatment with cyclophosphamide also did not influence resistance [38]. No quantitative or qualitative differences were found in active antibody synthesis in resistant and susceptible chickens [12].

Evidence for the involvement of cell mediated immunity in natural resistance was recently obtained in experiments with the late resistance model [39]. Chickens were immunosuppressed in the T cell functions by neonatal thymectomy and total body gamma irradiation and challenged with MDV at 8 weeks, an age level when natural resistance was expected to be well developed. Thymectomised irradiated chickens, deficient in T cell functions, succumbed to progressive tumor formation, whereas untreated hatchmates were resistant. In other studies, chickens resistant at hatching were similarly found to be less resistant if they were thymectomised prior to virus challenge [22, Sharma, unpublished].

The only evidence to date of the involvement of immune systems in HVT vaccine-induced immunity is that of Purchase and Sharma [28]. These authors found that HVT failed to provide protection in chickens pretreated with high doses of cyclophosphamide. Although the specific immune system was not delineated in the above study, subsequent observations that bursectomy did not influence vaccine protection [10] indicated that the lack of protection in cyclophosphamide treated chickens was most probably mediated through the transient suppressive effect of the drug on the T cell system during early stages of treatment [16, 31]. Chickens were vaccinated with HVT at 6 days of age, only 2 days after the last dose of cyclophosphamide was administered. Additional studies are obviously needed to firmly establish the influence of T cell suppression on vaccine protection. However, data on hand strongly suggest that cell mediated immune responses may be of principal importance both in natural and in vaccine induced resistance.

**ANTIGEN(S) INVOLVED IN PROTECTIVE IMMUNITY**

Several virus-related antigens have been detected in cells infected with MDV. Antigens A, B and C were recognised in immunodiffusion tests [7]. Chen and Purchase recognised a membrane antigen by immune fluorescense on the surface of viable monolayer cells infected with MDV [5]. Early and late antigens similar to those described for the Burkitt’s lymphoma cell lines have also been found in a lymphoblastoid cell line (MSB-1 line) [1] developed from
MD lymphoma [18]. Which of these antigens induces protective immunity against clinical MD is not yet known. Of much current interest is the tumor antigen (MATSA, Marek’s disease tumor-associated surface antigen) present in a certain proportion of lymphoma cells in vivo and in cells of MD lymphoblastoid cell lines [24, 45]. This antigen is not associated with lytic infection and is considered a marker for cells transformed by MDV.

Immunising chickens by repeated injection of gluteraldehyde-fixed cells of MD lymphoblastoid lines resulted in at least partial protection against challenge with virulent MDV [25]. This protection was attributed to immunity against MATSA. It is likely that in resistant chickens initial lesion development initiates a strong immune response against MATSA which mediates lesion regression and also arrests subsequent transformation. Because HVT inoculated birds also developed transient lymphoproliferative lesions that may contain MATSA bearing cells [44] anti-MATSA immunity may also play a role in HVT immunity. On the other hand, protection against MD has also been achieved by immunising chickens with MATSA-free cellular fractions obtained by disrupting monolayer cells lytically infected with HVT [14, 15]. Furthermore, protection by HVT vaccine against early reticuloendothelial cell proliferative phase of MDV infection [44] also indicates that anti-viral response must be important in vaccine immunity. It is likely that protective cell-mediated immunity may occur against MATSA as well as against other virus-related antigens and that the role of all these antigens in immune surveillance in MD may not be mutually exclusive.

Clearly a better assessment of the specific cell-mediated responses in chickens infected with MDV is needed to delineate the role played by various antigens. Some of the attempts to develop in vivo and in vitro tests to measure cell-mediated immunity in MD are of particular interest.

CELL-MEDIATED IMMUNITY IN MD

Certain crude antigenic fractions obtained from cells lytically infected with MDV or a partially purified “A” antigen preparation induced a local cutaneous delayed type hypersensitivity (DTH) reaction in chickens undergoing MDV infection [2, 11]. These in vivo studies suggested that chickens develop a detectable cellular response to virus-induced antigens. The “A” antigen preparation also caused a specific in vitro inhibition of radial migration of lymphocytes obtained from MDV infected chickens [11]. Crude antigen preparations, however, gave non-specific inhibition of lymphocytes from normal, control chickens. The migration-inhibition test has not been used widely as a measure of cell-mediated immunity in MD, presumably because of practical limitation such as the need for purified antigens and the complexity of the test procedure. To date, no reproducible in vitro assay procedures have been developed to quantitate cell-mediated immune response to viral antigens in MD. The development of such procedures is crucial for illucidating the role of these antigens in resistance to MD.

The availability of MD lymphoblastoid cell lines [1, 24] has, for the first time, provided the opportunity to utilize line cells to study in vitro immune response to antigens present on these cells. Recently we detected a specific cell-mediated cytotoxic response in chickens infected with MDV by a cytotoxicity test using MSB-1 cells as the target cells [40]. This cytotoxic response, presumably directed against MATSA, was detected 1–2 weeks after virus inoculation and paralleled the appearance of early lymphoproliferative lesions. The cytotoxic response lasted briefly and disappeared as lymphoproliferation intensified and progressed into
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gross lesion formation. Although specificity of this response to MATSA and the role it might play in resistance to MD have not yet been fully determined, the availability of reproducible cytotoxicity tests such as the one used by us should facilitate in vitro assessment of some aspects of cell-mediated immunity in MD.

REFERENCES


