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H2 Gene Control and Biological Activities of a T-Cell Mitogen Derived from *Mycoplasma arthritidis:* A Review

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Mycoplasma arthritidis generates a soluble, non-dialysable, polyclonal mitogen which is active for murine and human T lymphocytes in the presence of an adherent, radio-resistant, Ia-bearing accessory cell population. Genetic analysis has established that the I-E sub-region of the murine H2 gene complex controls responses to the mitogen and that this control is exercised at the level of the Ia-bearing accessory cell. Lymphocyte proliferation, induction of cytotoxic lymphocytes, and interferon induction are all under Ir gene control and appear to be dependent upon binding of the mitogen to a specific Ia antigen present on a subset of splenic cells. This mycoplasma mitogen provides a new model system to define the mechanisms of Ir gene control of lymphocyte activation.

INTRODUCTION

Microbial mitogens are receiving increasing attention as possible contributors to the pathogenesis of disease and to altered immune responses. Many mycoplasmas are known to exert mitogenic effects on normal unsensitized lymphocytes from a variety of hosts [1,2,3]. Whereas it is the murine B-cell subpopulation which responds to *M. pneumoniae, A. laidlawii,* and *M. neurolyticum* [4,5,6], both B cells and T cells respond to *M. pulmonis* [7]. Human T cells were also reported to be stimulated by *A. laidlawii* [5] and *M. pneumoniae* [8]. Emphasis in our laboratories has been on lymphocyte interactions with *M. arthritidis.* Since this organism is a potent arthritogenic agent of mice, rats, and rabbits [9], detailed study of its lymphocyte-activating properties may provide clues as to the mechanisms of pathogenesis of the diseases induced.

In this review I will summarize current knowledge concerning the T-lymphocyte-activating component of *M. arthritidis,* the cellular collaborations required, and the genetic control of T-lymphocyte activation. In addition, other properties of the mitogen, including macrophage activation and ability to induce interferon, will also be described. Detailed methodology can be found in the cited references. The genetic studies are summarized in Table 1.

RESULTS

Cellular Requirements for T-Cell Activation of M. arthritidis

The first evidence that *M. arthritidis* might produce a T-cell activator was in our observation that viable organisms induced splenic cells to become cytotoxic for

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TABLE 1
Genetic Control of Lymphocyte Responses to *M. arthritidis* Mitogen (MAS)

Source of Mouse Splenic Cells	Haplotypes Expressed at H2 Gene Complex I								Presence or Absence of Ia7[a]	Lymphocyte Responses to MAS			
	K	A	B	J	E	C	S	D		Cytotoxic T Cells	T-Cell Proliferation	Binding of the Mitogen	Interferon Production
Inbred Strains													
CBA, C3H	k	k	k	k	k	k	k	k	+	+	+	+	+
Balb/c	d	d	d	d	d	d	d	d	+	+	+	+	+
C57BL/10	b	b	b	b	b	b	b	b	−	−	−	−	−
Congenic Strains													
C3H.SW	b	b	b	b	b	b	b	b	−	−	−	−	−
B10.BR	k	k	k	k	k	k	k	k	+	+	+	+	+
B10.D2	d	d	d	d	d	d	d	d	+	+	+	+	+
B10.Q	q	q	q	q	q	q	q	q	−	NT[b]	−	−	−
Recombinant Strains													
B10.A (3R)	b	b	b	b	k	d	d	d	+	+	+	+	+
B10.A (4R)	k	k	b	b	b	b	b	b	−	−	−	−	−
B10.A (5R)	b	b	b	k	k	d	d	d	+	+	+	+	+
B10.A (18R)	b	b	b	b	b	b	b	d	−	−	−	−	−
BSVS	s	s	s	s	s	s	d	d	−	−	−	−	NT
A.TFR4	f	?	?	?	?	?	s	d	−	NT	−	−	−
A.TFR5	f	f	?	?	k	k	k	d	+	NT	+	+	+

[a]The Ia7 specificity is borne on the α chain of the I-E coded molecule.
[b]Not tested

syngeneic and allogeneic fibroblast targets [10]. Furthermore, cytotoxic activity was abolished by removing T cells by treatment with anti-Thy 1 antibody and complement [11]. Interestingly, non-viable washed *M. arthritidis* preparations were without significant T-cell activating properties. We also demonstrated that viable *M. arthritidis* was mitogenic for murine splenic cells, providing that multiplication of the organisms was controlled by antibiotics or that excess arginine was incorporated into culture media [1]. Lymphocyte proliferation was abolished by elimination of the T cells. Removal of adherent splenic cells by passage over nylon columns also abolished reactivity, suggesting a role for the macrophage or dendritic cell. We finally demonstrated that purified T cells were only activated by MAS in the presence of an adherent, Ia antigen-bearing, radio-resistant, accessory cell population [12].

Further study on the mitogenic moiety of *M. arthritidis* showed that the active component was present in supernatants from *M. arthritidis* broth cultures (MAS). Mitogenic activity was shown to be non-sedimentable for 15 hours at 110,000 *g*, non-dialysable, partially labile to 56°C for one hour, and inactivated by sepharose-bound protease [12]. The polyclonal nature of the T-cell mitogen has now been established [Gurish, Cole, Daynes: J Immunol, in press]. These results are important since the existence of a soluble mitogen indicates that mycoplasmas can activate lymphoid cells without the necessity for cell-cell interactions. The mitogen was also active for rat and human lymphocytes but not for rabbit, guinea pig, ovine, or bovine lymphocytes [13]. Human T lymphocytes also required an accessory cell population [14].

Ir Gene Control of Lymphocyte Activation

During this time, experiments were also being conducted to determine whether lymphocytes from different strains of mice reacted differently to the lymphocyte-activating properties of *M. arthritidis*. Using both proliferation and cytotoxicity assays with viable *M. arthritidis* and *M. arthritidis* supernatants (MAS), we established that lymphocytes from inbred mice of the k haplotype, i.e., CBA and C3H, and of the d haplotype, i.e., Balb/c, are responsive to *M. arthritidis*, whereas lymphocytes from b haplotype mice, i.e., C57BL/6 or C57BL/10, are non-responsive [15]. Furthermore, lymphocyte reactivity was shown to be dependent upon the haplotype expressed at the H2 gene complex. This conclusion was reached since congenic mouse strains, which differed from the parental strain only in the haplotype expressed at H2, always responded according to the haplotype at H2, and not the background haplotype. By using recombinant mice which express different haplotypes at different regions of the H2 complex, we identified the I-E sub-region as responsible for the genetic control of activation [15,16] (Table 1).

The demonstration of I-E region control for the mycoplasma mitogen appears to be unique. Although differences in the degree of responsiveness of lymphocytes from various mouse strains to the T-cell mitogens concanavalin A (Con-A) and phytohemagglutinin (PHA) have been identified, the genetic control of these differences appears to lie outside the H2 gene complex [17].

The availability of responder and non-responder mouse strains provided an opportunity to determine whether the genetic control of lymphocyte activation was mediated at the level of the T cell or the accessory cell. We showed that purified T cells from Fl hybrids between responder (C3H) and non-responder (C57BL/6) mice were activated by MAS in the presence of accessory cells from the hybrid or the C3H mice but were not stimulated in the presence of accessory cells from C57BL/6 mice.

In contrast, T-cell responses to Con-A were supported by accessory cells from all strains of mice. Thus, the accessory cell appears to be responsible for the genetic control of lymphocyte reactivity to MAS [12].

Mitogen Binding to Lymphocyte Surfaces

The next phase of the work was designed to study the interaction of the mitogen with splenic cells. A key question was to determine whether the mitogen bound only to responsive lymphocytes or whether, as for the plant mitogens, it bound to all lymphoid cells. A 1:250 dilution of MAS was absorbed twice at 37°C for one hour with 5×10^7 splenic lymphoid cells from CBA or C57BL/10 mice. Cells were removed by centrifugation and the supernatants assayed at a 1:5 dilution for lymphocyte proliferation, using fresh CBA splenic cells. Whereas unabsorbed MAS induced marked proliferation of lymphocytes, treatment with responsive CBA spleen cells resulted in a virtual loss of mitogenic activity. In contrast, MAS absorbed with non-responsive C57BL/10 splenic cells retained full activity [18]. Lesser concentrations of responsive lymphocytes only partially removed the activity.

Similar absorption experiments using spleen cells from congenic and appropriate background mice established that the ability to absorb the mitogen was dependent upon the H2 gene complex. By using recombinant mice, we further demonstrated that mitogen binding was dependent upon the haplotype expressed at the I-E subregion of the murine H2 gene complex and appeared to associate with the presence of the Ia7 serological specificity. To confirm the latter association, we compared binding of the mitogen to splenic cells from two strains of mice (A.TFR5 and A.TFR4) which differed in Ia antigen expression only in the presence or absence of Ia7, respectively. Only those lymphocytes which genotypically express Ia7 bound the mitogen [18]. However, binding to A.TFR5 splenic cells was much less than that seen with either CBA, C3H, or Balb/c cells [18]. The lesser binding may correlate with the poor phenotypic expression of Ia7 on A.TFR5 cells [19]. Most I-E region-coded molecules consist of an α and a β chain. Since the Ia7 specificity when present is borne on the α chain of the molecule and since the β chain is absent from A.TFR5 mice [20], our results imply that mitogen binding is associated with the presence of the α chain of the I-E molecule.

Thus, there appears to be complete correlation between the ability of MAS to activate lymphocytes and to bind the splenic cells (Table 1). An attractive hypothesis for these findings is that an Ir region product, i.e., an Ia antigen, acts as the receptor site for the mitogen. A number of experiments were conducted to address this issue. First, we compared the ability of the mitogen to bind to various lymphocyte subpopulations of CBA mice. Splenic cells depleted of T cells by treatment with anti-Thy 1 antiserum and complement were just as effective in mitogen removal from MAS as were spleen cells treated with complement alone. Thymocytes were ineffective in removing the mitogen, confirming that the T cell is not the binding cell. However, treatment of splenic cells with syngeneic anti-Ia antiserum (ATH anti-ATL) and complement markedly reduced their binding capacity. Other experiments have shown that glass-adherent cells are also capable of binding the mitogen. Further evidence for the role of Ia antigens comes from studies in which we demonstrated that syngeneic anti-Ia antiserum, in the absence of complement, blocked proliferative responses to MAS but was minimally effective toward responses to PHA and Con-A [18]. The combined data strongly suggest that the mitogen binds to Ia-bearing cells which are then able in some way to present it to the T cell. The future use of isolated

Ia antigens and monoclonal antibodies directed against specific I-E region determinants are now required to identify definitively the precise binding site on the I-E coded molecule.

Macrophage Activation by M. arthritidis

Since the Ia-bearing adherent cells, i.e., macrophages or dendritic cells, play a key role in T-lymphocyte responses to MAS, experiments were undertaken to determine whether *M. arthritidis* exhibited other effects on macrophage functions. Evidence that some mycoplasmas activate macrophages has been obtained by Hibbs [21] and Taylor-Robinson et al. [22]. However, since peritoneal macrophages were used, the activation seen might have been mediated by lymphokine activity, generated by activation of contaminating lymphocytes. To avoid this problem, our studies were performed using the J774.1 continuous macrophage line which originated from the responder Balb/c ($H2^d$) mouse strain.

First, we determined whether either viable *M. arthritidis* or MAS induced morphological changes. Whereas untreated macrophages, treated with uninoculated mycoplasma broth, exhibited a uniform smooth appearance, those exposed for 24 hours to viable *M. arthritidis* or MAS showed changes characteristic of those induced by BCG cell walls. Thus, the macrophages developed enhanced spreading, pseudopod-like extensions of the membrane and numerous endocytic vesicles and phagosomes [23]. Additional studies showed that macrophages treated with viable *M. arthritidis* or MAS and extensively washed exhibited similar changes in function to those induced with BCG cell walls, i.e., enhanced listericidal activity, acquisition of tumoricidal properties, enhanced production of acid phosphatase, and increased uptake of ^{14}C glucosamine [23]. It remains to be determined whether the macrophage-activating component in MAS is in fact the mitogenic moiety. Mitogen purification studies now under way will eventually answer this question. The mechanisms of macrophage activation and the role of mycoplasmas to alter continuous line macrophage functions poses yet another potential pitfall for the cell biologist unaware of the presence of these contaminants.

Interferon Induction by M. arthritidis

Previous studies from our laboratories indicated that mycoplasmas induced interferon *in vivo* in mice and in sheep and human lymphocyte cultures [24,25,26]. These and recent studies by other investigators [27] suggested that the interferon produced was α interferon. Alpha interferon is characteristically induced by viruses and B-cell mitogens, whereas T-cell mitogens characteristically induce immune or γ interferon. Thus our new finding that *M. arthritidis* produces a T-cell mitogen is paradoxical in view of the ability of this organism to induce α interferon in mice.

Preliminary studies presented at this conference [28] and now in press [Cole, Thorpe: J Immunol, 1983] indicate that MAS is in fact an inducer of human γ interferon. We also investigated this issue using CBA mouse lymphocyte cultures with MAS. Interferon was detected after 24 hours and peaked after three to four days. The anti-viral substance was subjected to the usual criteria for acceptance as an interferon and, furthermore, was shown to be pH labile and neutralized by anti-γ interferon antibody but not by anti-α interferon antibody. By comparing the interferon response of lymphocytes from various inbred, congenic, and recombinant mice we further demonstrated a complete correlation between the ability of MAS to

induce interferon and its ability to induce lymphocyte proliferation. Thus the induction of γ interferon by MAS is also under Ir gene control (Table 1).

DISCUSSION

There are many important implications which arise from these studies. First, the *M. arthritidis* mitogen represents a new tool to examine the mechanisms of Ir gene control of lymphocyte activation. Ia antigens, the proposed products of Ir genes, are considered to play a pivotal role as recognition markers for the cell collaborations necessary for generation of humoral and cellular immune responses. Our system suggests that Ia antigens might serve as receptor sites for T-lymphocyte-activating substances.

Second, since responder and non-responder mouse strains are available, we have an ideal model to examine the role of mitogen-induced lymphocyte activation in various disease processes. The chronic inflammatory disease induced by many mycoplasmas is highly suggestive of a massive stimulation of cells of the immune system. In the case of *M. pulmonis,* evidence has been obtained that mitogen-containing extracts can induce pneumonic lesions [29].

Third, these studies have also shown that γ interferon induced by a microbial mitogen can also be under Ir gene control. One implication of this new finding is that this reaction may represent a potentially potent genetic control mechanism for susceptibility to infectious disease. Thus, γ interferon, which is known to act synergistically with α interferon [30], is becoming recognized as a powerful modulator of immune response and host defenses; *M. arthritidis* and some bacteria do in fact induce both α and γ interferons.

It remains to be determined whether the genetic control of the T-cell mitogen of *M. arthritidis* represents a unique system or whether other mycoplasmas or bacteria will eventually be shown to possess similarly acting mitogens. Studies by Stanbridge et al. [31] using whole *M. hyorhinis* organisms indicated differences in the degree of proliferation of lymphocytes from different recombinant mouse strains. Differences in the degree of proliferation of LEW and F344 rat lymphocytes to *M. pulmonis* and other mitogens have also been reported at this conference [32]. These studies should be continued and the genetic control mechanisms identified.

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REFERENCES

1. Cole BC, Aldridge KE, Ward JR: Mycoplasma-dependent activation of normal lymphocytes: mitogenic potential of mycoplasmas for mouse lymphocytes. Infect Immun 18:383-399, 1977
2. Naot Y, Tully JG, Ginsburg H: Lymphocyte activation by various mycoplasma strains and species. Infect Immun 18:310-317, 1977
3. Ginsburg H, Nicolet J: Extensive transformation of lymphocytes by a mycoplasma organism. Nature (New Biol) 246:143-146, 1973
4. Biberfeld G, Gronowicz E: Mycoplasma pneumoniae is a polyclonal B-cell activator. Nature 261:238-239, 1976
5. Kirchner H, Brunner H, Ruhl H: Effect of *A. laidlawii* on murine and human lymphocyte cultures. Clin Exp Immunol 29:176-180, 1977

6. Naot Y, Merchav S, Ginsburg H: *Mycoplasma neurolyticum*: a potent mitogen for rat B lymphocytes. Eur J Immunol 9:185–189, 1979
7. Naot Y, Merchav S, Ben-David E, et al: Mitogenic activity of *Mycoplasma pulmonis*. I. Stimulation of rat B and T lymphocytes. Immunology 36:399–406, 1979
8. Biberfeld G: Activation of human lymphocyte subpopulations by *Mycoplasma pneumoniae*. Scan J Immunol 6:1145–1150, 1977
9. Cole BC, Ward JR: Mycoplasmas as arthritogenic agents. In The Mycoplasmas, Vol II. Edited by MF Barile, S Razin, JG Tully, et al. New York, Academic Press, 1979, pp 367–398
10. Aldridge KE, Cole BC, Ward JR: Mycoplasma-dependent activation of normal lymphocytes: induction of a lymphocyte-mediated cytotoxicity for allogeneic and syngeneic mouse target cells. Infect Immun 18:377–385, 1977
11. Cole BC, Aldridge KE, Sullivan GJ, et al: Mycoplasma-dependent activation of normal mouse lymphocytes: requirement for functional T-lymphocytes in the cytotoxicity reaction mediated by *Mycoplasma arthritidis*. Infect Immun 30:90–98, 1980
12. Cole BC, Sullivan GJ, Daynes RA, et al: Stimulation of mouse lymphocytes by a mitogen derived from *Mycoplasma arthritidis*. II Cellular requirements for T cell transformation mediated by a soluble mycoplasma mitogen. J Immunol 128:2013–2018, 1982
13. Cole BC, Washburn LR, Sullivan GJ, et al: Specificity of a mycoplasma mitogen for lymphocytes from human and various animal hosts. Infect Immun 36:662–666, 1982
14. Daynes RA, Novak JM, Cole BC: Comparison of the cellular requirements for human T-cell transformation by a soluble mitogen derived from *Mycoplasma arthritidis* and concanavalin A. J Immunol 129:936–938, 1982
15. Cole BC, Daynes RA, Ward JR: Mycoplasma-mediated activation of normal mouse lymphocytes: induction of cytotoxic lymphocytes and lymphocyte transformation by Mycoplasma arthritidis are under Ir gene control. J Immunol 126:922–927, 1981
16. Cole BC, Daynes RA, Ward JR: Stimulation of mouse lymphocytes by a mitogen derived from *Mycoplasma arthritidis*. I. Transformation is associated with an H-2-linked gene that maps to the I-E/I-C sub-region. J Immunol 127:1931–1936, 1981
17. Williams RM, Benacerraf B: Genetic control of thymus-derived cell function. I. *In vitro* DNA synthetic response of normal mouse spleen cells stimulated by the mitogens concanavalin A and phytohemagglutinin. J Exp Med 135:1279–1292, 1972
18. Cole BC, Daynes RA, Ward JR: Stimulation of mouse lymphocytes by a mitogen derived from *Mycoplasma arthritidis*. III. Ir gene control of lymphocyte transformation correlates with binding of the mitogen to specific Ia-bearing cells. J Immunol 129:1352–1359, 1982
19. Murphy DB, Jones PP, McDevitt MO: Serological analysis of recombinant strains A.TFR5. In Ir Genes and Ia Antigens. Edited by MO McDevitt. New York, Academic Press, 1978, p 37
20. Lafuse WP, McCormick JF, David CS: Lack of gene complementation prevents the expression of $I\text{-}E^k$ in recombinant A.TFR5. J Immunol 124:2511–2513, 1980
21. Hibbs JB: Evidence for activation of macrophages and modification of target cells by in vitro association with mycoplasmas. Clin Res 21:212, 1973
22. Taylor-Robinson D, Schorlemma HU, Furr PM, et al: Macrophage secretion and the complement cleavage product C3a in the pathogenesis of infections by mycoplasmas and L-forms of bacteria and in immunity to these organisms. Clin Exp Immunol 33:480–494, 1978
23. Dietz JN, Cole BC: Direct activation of the continuous J774.1 macrophage cell line by *Mycoplasma arthritidis*. Infect Immun 37:811–819, 1982
24. Cole BC, Overall JC Jr, Lombardi PS, et al: Induction of interferon in ovine and human lymphocyte cultures by mycoplasmas. Infect Immun 14:88–94, 1976
25. Rinaldo CR, Cole BC, Overall JC Jr, et al: Induction of interferon in mice by mycoplasmas. Infect Immun 10:1296–1301, 1974
26. Rinaldo CR, Overall JC Jr, Cole BC, et al: Mycoplasma-associated induction of interferon in ovine leukocytes. Infect Immun 8:796–803, 1973
27. Beck J, Brunner H, Marcucci R, et al: Induction of interferon by mycoplasmas in mouse spleen cultures. J Interferon Res 2:31–36, 1982
28. Cole BC, Thorpe RN: Induction of human γ interferon by a mitogen derived from *Mycoplasma arthritidis* and its inhibition with monoclonal antibodies to HLA.DR. Proceedings of the Fourth IOM Congress, Tokyo. Abstracts.
29. Naot Y, Davidson S, Lindenbaum ES: Mitogenicity and pathogenicity of *Mycoplasma pulmonis* in rats. I. Atypical interstitial pneumonia induced by mitogenic mycoplasmal membranes. J Infect Dis 143:55–62, 1981

30. Baron S, Blalock JE, Dianzani F, et al: Immune interferon: Some properties and functions. Bull NY Acad Sci 350:130–144, 1976
31. Stanbridge EJ, Bretzius KA, Good RF: Mycoplasma-lymphocyte interactions: Ir gene control of mitogenesis and a paradoxical interaction with Thy-1 bearing cells. Isr J Medical Sci 17:628–632, 1981
32. Davis JK, Thorp RB, Simeka JW, et al: Nonspecific mitogenic responses of LEW and F344 rat lymphocytes toward *Mycoplasma pulmonis*. Proceedings of the Fourth IOM Congress, Tokyo. Abstracts. Yale J Biol Med 56:914, 1983