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I-E/I-C Region-Associated Induction of Murine Gamma Interferon by a Haplotype-Restricted Polyclonal T-Cell Mitogen Derived from *Mycoplasma arthritidis*

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Cell-free supernatants from cultures of *Mycoplasma arthritidis* induced significant levels of interferon when cocultured with murine splenic cells. On the basis of physicochemical characteristics and antibody neutralization studies, the antiviral substance was identified as gamma interferon. Use of inbred and congenic mouse strains established that splenic cells from mice expressing the $H2^k$ and $H2^d$ haplotypes produced interferon in response to *M. arthritidis* culture supernatants, but those from mice with $H2^b$ and $H2^q$ haplotypes did not. Further studies with recombinant mouse strains established that interferon induction by the mycoplasma supernatant was associated with the haplotype expressed at the I-E/I-C subregion of the murine major histocompatibility complex. The specificity seen for interferon induction was identical with that reported earlier for induction of cytotoxic lymphocytes and for lymphocyte proliferation in response to the mitogen. All of these reactions appear to be dependent upon binding of the mitogen to specific I-E/I-C region-coded products present on splenic cell surfaces. The observations presented introduce the concept that microbial mitogens or their lymphokine products might modify immune responses and defense mechanisms of the naive host in a genetically restricted manner.

Mycoplasmas, many of which are common agents of chronic joint, respiratory, and urogenital disease (30), have been shown to be inducers of interferon (IFN) (3, 4, 11, 25–27). Although IFNs were originally considered to be primarily antiviral agents, they are now recognized to have important biological and immunoregulatory activities (31). IFN of the type induced by viruses or B-cell mitogens (IFN-α) is produced when intact mycoplasmas or mycoplasma membrane extracts are added to lymphoid cell cultures or inoculated into experimental animals. IFN-γ is induced by T-cell mitogens or by antigen-specific mediated T-cell proliferation.

Mycoplasma arthritidis, an agent of rat, mouse, and rabbit arthritis (14), has been shown to induce mouse T lymphocytes to become cytotoxic for syngeneic and allogeneic fibroblast target cells (1) and to undergo blast transformation (7). T-cell activation of murine lymphocytes by *M. arthritidis* has been shown to be due to a soluble component present in culture supernatants (12), which has been recently shown to be a polyclonal T-cell mitogen (35). Mitogen-containing supernatants have also been demonstrated to directly activate continuous line macrophages (18). Stimulation of both murine and human T lymphocytes requires the presence of accessory cells bearing Ia (immune-associated) antigens (12, 17). Ia antigens, the proposed products of immune response genes, are believed to play a key role in the control of cell collaborations, which are necessary for activation of the immune system. Our finding that lymphocyte transformation induced by the T-cell mitogen of *M. arthritidis* is restricted to certain mouse strains allowed us to map control of the reaction to the I-E/I-C subregion of the murine H2 histocompatibility complex (*H2* gene complex) (8). Furthermore, evidence has been obtained recently that T-lymphocyte transformation was dependent upon binding of the mitogen to specific immune response gene products, presumably the Ia antigens, on accessory cell surfaces (9).

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Since all previous work suggested that mycoplasmas, including *M. arthritidis*, induced a pH-stable IFN-α (3, 4, 11, 25–27), we therefore investigated IFN induction by the T-cell mitogen of *M. arthritidis*. These studies were considered important as a potential model for IFN-γ production, since the precise cellular and genetic requirements for induction of gamma IFNs by antigens and mitogens are much less well established than are the requirements for lymphocyte proliferative responses (29, 34). In this report, we demonstrate that the *M. arthritidis* T-cell mitogen is an inducer of IFN-γ and that the haplotype specificity of the reaction is identical to that seen for lymphocyte proliferation and induction of cytotoxic lymphocytes. The results also imply that a common initial event, namely, binding of the mitogen to specific I region-coded molecules, is necessary for both of these reactions.

MATERIALS AND METHODS

MAS. *M. arthritidis* culture supernatants (MAS) were prepared with *M. arthritidis* 14124 P10 (19). The organisms were grown in a simplified dialysate medium based upon that described by Kenny (23, 24) as detailed previously (9) with the addition of a boiled serum supplement. Supernatants were prepared from log-phase cultures of 10^7 to 10^8 CFU per ml by centrifugation at 27,000 × *g* for 30 min followed by successive filtration through one 0.45-μm and three 0.22-μm membrane filters (Millipore Corp., Bedford, Mass.). Supernatants were dialyzed overnight against pH 7.2 phosphate-buffered saline, cultured on mycoplasma agar (5) and on nutrient agar to ensure absence of viable organisms, and then stored in portions at −70°C until use.

Animals and animal screening. Jack Stimpfling (Great Falls, Mont.) kindly supplied the breeding stock of C57BL/10, B10.D2, B10.Q, B10.BR, B10.A (3R), B10.A (4R), B10.A (5R), and B10.A (18R) mice. Cella S. David graciously donated the breeding stock of A.TFR4 and A.TFR5 mice. The breeding stock of BALB/c was pur-

chased from Charles River Breeding Rivers Laboratories, Inc., Wilmington, Mass. CBA/J mice were purchased directly from Jackson Laboratories, Bar Harbor, Maine. C3H and C3H.SW mice were obtained both from Jackson Laboratories and from stock raised by R. A. Daynes, University of Utah, Salt Lake City. Breeding stocks were generously raised in the laboratories of R. A. Daynes.

Representative mice of each strain and source were screened by enzyme-linked immunosorbant assay to ensure absence of preinfection with *M. arthritidis* as detailed previously (33). Absorbances of test sera at 1:10 dilutions were all within less than 3 standard deviations of the absorbances obtained with control sera.

Mice of either sex up to 12 months old were used as donors of splenic lymphocytes. No association was seen between IFN levels and the sex or age of donor mice.

Induction of IFN. The spleens of mice sacrificed by cervical dislocation were minced in prewarmed RPMI 1640 medium containing 2 mM L-glutamine and 200 U of penicillin G per ml. Cell suspensions were passed through sterile 80-mesh screens, pelleted at 200 × *g* for 5 min, treated with warm 0.83% (wt/vol) NH_4Cl buffered to pH 7.2 with 60 mM Tris to remove erythrocytes, repelleted, and finally suspended in complete RPMI medium with 5% (vol/vol) fetal bovine serum (Hyclone; Sterile Systems, Inc., Logan, Utah). Viable cells were enumerated by trypan blue exclusion and consistently exceeded 95%.

Spleen cell suspensions were adjusted to 2.5×10^6 cells per ml in complete RPMI 1640 medium and were distributed in 0.5- or 1.0-ml amounts in culture tubes (12 by 75 mm) or in 0.2-ml amounts in flat-bottomed culture plates (Falcon Plastics, Oxnard, Calif.). IFN inducers were added in volumes 1/10 of the total cultures to give final concentrations of 1:25 to 1:100 MAS, 5 to 10 μg of phytohemagglutinin (PHA; HA16; Burroughs Wellcome, Research Triangle Park, N.C.) per ml, 3.6 μg of concanavalin A (ConA; Sigma Chemical Co., St. Louis, Mo.) per ml, and a 1:100 or 1:500 dilution of a UV-irradiated preparation of Newcastle disease virus (NDV). The original allantoic pool of NDV exhibited 2×10^8 PFU/ml and 6×10^3 hemagglutinating units per ml. After UV irradiation with a Sylvania germicidal bulb (G15T8) at a distance of 7.13 cm for 30 min, the allantoic pool contained <3 PFU/ml. Negative controls for IFN consisted of spleen cell suspensions supplemented with 1/10 volume of RPMI medium or suspensions treated with various dilutions of uninoculated mycoplasma medium.

Lymphocyte cultures were incubated for 1 to 5 days at 37°C in a humidified incubator with 5% (vol/vol) CO_2 in air. At harvest, the spleen cells were pelleted at 400 × *g* for 10 min, and supernatants were stored at −70°C until assayed for IFN.

IFN. Mouse L-929 cells were maintained without antibiotics and were regularly screened to ensure absence of mycoplasma infection by using both direct culture and DNA-staining techniques (6). The microtiter method for IFN assay was modified from that described by Green et al. (20). Mouse L-929 cells (7×10^5 per ml) suspended in RPMI 1640 medium containing 2% (vol/vol) fetal calf serum were seeded in 0.1-ml volumes in flat-bottomed 96-well culture plates (Microtest II; Falcon Plastics) and incubated in a CO_2 incubator until confluency (16 to 24 h). Duplicate portions (50 μl) of each IFN sample were then added to the first row of wells, and serial threefold dilutions were made with an automatic diluter fitted with 12 50-μl calibrated loops (Titertek Medimixer; Flow Laboratories, Inc., Rockville, Md.). Control cells and cells treated with IFN were incubated for 6 h. Supernatants were removed and the monolayers were washed. Encephalomyocarditis virus was then added in 25-μl amounts to give 25 to 35 PFU per well. Wells receiving no virus served as additional controls. After absorption for 1 h at 37°C, 0.1 ml of RPMI medium with 2% (vol/vol) fetal calf serum was added to each well, and incubation was continued until virus plaques were visible (18 to 24 h). The cell monolayers were then fixed with 10% (vol/vol) Formalin for 30 min, washed under running tap water, and stained with 1% (wt/vol) crystal violet in 70% (vol/vol) ethanol. Titers of IFN were calculated as the reciprocal of the highest dilution of IFN which inhibited 50% of the plaques present in the absence of IFN. All assays included a known reference L-cell IFN standard (IFN-α/β; NIH no. G002-904-511).

Antibody neutralization of IFN. A specific antibody to mouse IFN-α/β (NIH no. G-024-501-568) and an antibody to mouse IFN-γ (kindly supplied by M. P. Langford, University of Texas, Galveston) were each used at a dilution that consistently reduced 50 U of L-cell IFN-α/β standard or a laboratory produced PHA IFN-γ standard, respectively, by 90%. Test samples of IFN were diluted, when necessary, to contain approximately 50 U of IFN. The assay conditions for IFN were as described above, except that a 1-h treatment of the IFN samples at 20°C with each antibody preceded the IFN titration. In addition, the assay utilized twofold rather than threefold dilutions of the samples. Controls included the IFN test samples and standards without antibody treatment.

RESULTS

Preliminary studies established that MAS induced peak levels of 243 U of IFN in C3H spleen cells after 3 days of incubation, whereas cells treated with uninoculated mycoplasma medium failed to produce IFN (<3 U). Cells treated with PHA induced peak IFN levels of 312 U after 2 to 3 days, and those exposed to UV-irradiated NDV produced peak levels of 81 U after 1 day. Since NDV-induced IFN titers remained undiminished through 3 days, all future studies utilized a 3-day incubation period. Additional studies demonstrated that the maximal IFN response was achieved with 1:25 to 1:100 dilutions of MAS, 5 to 10 μg of PHA per ml, 3.6 μg of ConA per ml, and a 1:100 dilution of NDV (2×10^6 PFU/ml of a UV-irradiated pool).

In the first experiments we compared the ability of splenic cells from inbred CBA ($H2^k$), BALB/c ($H2^d$), and C57BL/10 ($H2^b$) mouse strains to produce IFN in response to MAS, PHA, ConA, and NDV. These strains were chosen since previous studies had established that lymphocytes from mice with $H2^k$ and $H2^d$ haplotypes underwent blast transformation in response to MAS, whereas those expressing $H2^b$ did not (8). The results (Table 1) established that splenic cells from CBA and BALB/c mice produced IFN in response to all inducers. In contrast, cells from C57BL/10 mice failed to produce IFN in the presence of all dilutions of MAS, yet they responded to PHA, ConA, and NDV.

The next series of experiments (Table 2) was conducted to determine whether the failure of C57BL/10 mice to induce IFN was associated with the haplotype expressed at the *H2* gene major histocompatibility complex (MHC) or with background genes. In the first experiment, the inbred C3H ($H2^k$) mice produced high levels of IFN (243 to 875 U) in response to MAS in contrast with the negative response (<3 U) of the congenic C3H.SW mice, which possessed identical background genes but a b haplotype at the MHC. In the second experiment, C57BL/10 ($H2^b$) mice failed to produce IFN in

TABLE 1. IFN production by splenic cells from inbred mouse strains in response to MAS

Expt	Source of splenic cells (haplotype)	Animal no.	IFN production (U) in response to:[a]						
			MAS			PHA (10 μg/ml)	ConA (3.6 μg/ml)	NDV (1:100)[b]	Control (no inducer)
			1:25	1:50	1:100				
1	CBA ($H2^k$)	1	11	19	7	150	6	3	<3
		2	27	14	14	130	27	<3	<3
		3	14	22	6	7	19	7	<3
	C57BL/10 ($H2^b$)	1	<3	<3	<3	59	68	35	<3
		2	<3	<3	<3	8	225	18	<3
		3	<3	<3	<3	7	14	8	<3
2	BALB/c ($H2^d$)	1	122	NT[c]	194	21	NT	8	<3
		2	74	NT	52	63	NT	45	<3
		3	54	NT	59	96	NT	41	<3

[a] IFN samples were collected after 3 days of incubation at 37°C. The values shown are mean values of duplicate titrations.
[b] The NDV dilution used contained 2×10^6 PFU/ml before UV irradiation (see the text).
[c] NT, Not tested.

response to MAS, yet the congenic B10.D2 mice, which had identical background genes but a d haplotype at the MHC, produced significant levels of IFN (8 to 54 U). With the exception of lymphocytes from one C57/BL10 mouse treated with PHA, all lymphocyte suspensions produced IFN when cocultured with PHA and NDV. The data suggest that, in contrast with other inducers, MAS-mediated IFN induction associates with the haplotype expressed at the murine MHC.

A large series of experiments was next conducted to confirm and extend the above observations. In addition, we also tested lymphocytes from recombinant mouse strains on a C57BL/10 background [B10.A(3R), B10.A(4R), B10.A(5R), B10.A(18R)], which differed only in the haplotype expressed at various subregions of the MHC. The results are expressed in Table 3 as the mean maximum IFN titers irrespective of the dose of the inducer. For comparative purposes, we also summarized previous data on the mitogenic potential of MAS (12) and on the binding capacity of the mitogen for the splenic cells tested (9).

Although IFN titers varied considerably, splenic cells from all mouse strains produced IFN in response to PHA and NDV. Splenic cells from inbred and congenic mice expressing the $H2^k$ and $H2^d$ haplotypes produced IFN in response to MAS, whereas those from mice expressing $H2^b$ or $H2^q$ did not. The results obtained with the congenic mice C3H.SW, B10.D2, and B10.BR support the previous data, suggesting a role for the MHC in determining IFN responses to MAS. Data obtained with the recombinant B10.A(4R) strain, which expresses the IFN-inducing k haplotype only at the K and I-A regions of the MHC, indicate that responsiveness is associated with a gene located to the right of the I-A subregion. Results with lymphocytes from B10.A(5R) and B10.A(3R) mice indicated that responses to MAS are dependent upon genes located to the right of the I-B subregion and to the right of the I-J subregion, respectively. Studies utilizing B10.A(18R) mice established that the controlling gene is located to the left of the D region, most probably in the I-E or I-C subregions which code for lymphocyte surface antigens.

Further examination of the results indicated that IFN induction by MAS correlated with the ability of the mitogen to induce lymphocyte proliferation and to bind to splenic cells of IFN-producing strains. Statistical examination of all data (Mann-Whitney rank sum analysis) indicated that there was a significant difference (>99.99%) in IFN levels produced by mouse lymphocytes which proliferated in response to MAS as compared with those which did not. Furthermore, lymphocytes from mouse strains responsive to MAS all express the Ia-7 serological specificity, whereas those nonresponsive do not (28). This association was confirmed

TABLE 2. IFN production by splenic cells from inbred and congenic mouse strains in response to MAS

Expt	Source of splenic cells (haplotype)	Animal no.	IFN production (U) in response to:[a]			
			MAS		PHA (10 μg/ml)	NDV[b] 1:100
			1:25	1:100		
1	C3H ($H2^k$)	1	875	243	486	37
		2	243	220	174	12
		3	596	135	171	20
	C3H.SW ($H2^b$)	1	<3	<3	41	23
		2	<3	<3	61	45
		3	<3	<3	51	13
2	C57BL/10 ($H2^b$)	1	<3	<3	9	21
		2	<3	<3	8	7
		3	<3	<3	<3	2,187
	B10.D2 ($H2^d$)	1	8	8	20	7
		2	54	7	243	20
		3	23	21	12	7

[a] IFN samples were collected after 3 days of incubation at 37°C.
[b] The NDV dilution used contained 2×10^6 PFU/ml before UV irradiation (see the text).

TABLE 3. Location of mouse gene(s) associated with induction of leukocyte IFN in response to MAS

Donor strain of splenic cells	No. tested	Haplotype expressed at *H2* gene complex[a] I region:								Mean maximum IFN titer with:[b]			Mitogenic response[c]		Binding of MAS[c]	Presence of Ia7
		K	A	B	J	E	C	S	D	MAS	PHA	NDV	MAS	PHA		
Inbreds																
CBA	9	k	k	k	k	k	k	k	k	130	77	13	+	+	+	+
C3H	8	k	k	k	k	k	k	k	k	289	147	28	+	+	+	+
BALB/c	7	d	d	d	d	d	d	d	d	57	30	15	+	+	+	+
C57BL/10	11	b	b	b	b	b	b	b	b	<3	9	270	−	+	−	−
Congenics																
C3H.SW	7	b	b	b	b	b	b	b	b	<3	35	19	−	+	−	−
B10.D2	4	d	d	d	d	d	d	d	d	24	69	42	+	+	+	+
B10.BR	3	k	k	k	k	k	k	k	k	122	55	34	+	+	+	+
B10.Q	2	q	q	q	q	q	q	q	q	<3	59	47	−	+	−	−
Recombinants																
B10.A(4R)	5	k	k	b	b	b	b	b	b	<3	55	83	−	+	−	−
B10.A(5R)	3	b	b	b	**k**	**k**	**d**	**d**	**d**	327	138	48	+	+	+	+
B10.A(3R)	3	b	b	b	b	**k**	**d**	**d**	**d**	320	185	75	+	+	+	+
B10.A(18R)	3	b	b	b	b	**b**	**b**	**b**	d	<3	5	14	−	+	−	−
A.TFR4	7	f	f	?	?	?	?	s	d	<3	25	124	−	+	−	−
A.TFR5	4	f	f	?	?	k	k	k	d	57	88	192	+	+	+	+

[a] Information summarized by Sheffler and David (28). Boldface regions indicate the location of the gene associated with IFN responses to MAS.

[b] Maximum IFN titers, irrespective of the dose of inducer, in response to MAS (1:25 to 1:100), PHA (5 to 10 μg/ml), or UV-irradiated NDV (1:100, 2×10^6 PFU). Spleen cells without inducer failed to produce IFN.

[c] Ability of splenic cells to undergo proliferation or to remove mitogenic activity from MAS after incubation and pelleting of cells. The data are detailed in references 8 and 9, respectively.

since splenic lymphocytes from A.TFR5 mice produced IFN in response to MAS whereas those from A.TFR4 mice did not. A.TFR5 and A.TFR4 mice differ in known serological specificities only in the absence of Ia-7 in the negatively responding A.TFR4 mice (28). Lymphocyte proliferation and binding of MAS were also associated with the presence of Ia-7 in these mice.

Considerable differences were noted in the levels of IFN induced by MAS in responsive mouse strains. Thus, lymphocytes from most strains expressing the k haplotype at the I-E subregion ($I\text{-}E^k$) [CBA, C3H, B10.BR, B10.A(3R), B10.A(5R)] produced higher titers to MAS than did mouse strains expressing $I\text{-}E^d$ (BALB/c, B10.D2). The experimental design, however, was unsuitable for statistical evaluation of these differences. Likewise, the significance of the variations in IFN levels produced in response to PHA and NDV cannot be established without further study.

Since previous studies have suggested that IFN induced by mycoplasmas, including *M. arthritidis*, was IFN-α on the basis of stability to a pH of 2.0, we conducted preliminary work to characterize the IFNs induced by MAS when cultured with C3H and BALB/c splenic cells. MAS- and PHA-induced interferons were found to be acid labile, whereas that induced by NDV was acid stable. Furthermore, specific anti-mouse IFN-γ antibody treatment resulted in a 95% or greater reduction in IFN levels induced by MAS and PHA but was ineffective against NDV-induced IFN-α. Antibody to IFN-α/β reduced titers of NDV-induced IFN by 75% but had no effect on that mediated by MAS. The results indicate that MAS is primarily an inducer of IFN-γ in cultured mouse splenic cells.

DISCUSSION

MAS were found to induce IFN-γ in cultures of murine splenic cells from strains which expressed the $H2^k$ or $H2^d$ haplotypes but not from those expressing $H2^b$ or $H2^q$. The use of recombinant mice established that the gene which determined IFN production was located within the I/E to S region of the MHC. Since the S region codes for serum proteins, we conclude that IFN responses to MAS are dependent upon the haplotype expressed at the I-E/I-C region. The results obtained were in full agreement with previous results indicating that lymphocyte proliferation in response to MAS and ability of the mitogen present to bind to splenic cells were also dependent on the I-E/I-C region of the murine MHC (8, 9). The above data and results obtained with A.TFR4 and A.TFR5 mice suggest that responsiveness to MAS is associated with expression of the Ia-7 serological specificity which is borne on the α chain of the I-E-coded molecule (15, 16, 22).

There are number of major implications to these studies. First, they suggest that IFN-γ production and T-lymphocyte proliferation mediated by MAS are associated with an initial event, namely, binding of the mitogen to Ia-bearing splenic cells. Previous data demonstrated that T-cell proliferation required Ia-bearing adherent accessory cells (12) and that the mitogen bound only to the Ia-bearing splenic cell subpopulation of responder haplotype mice (9). It remains to be determined, however, whether the actual IFN-producing cell is the same as that which undergoes proliferation. A number of other studies indicate that proliferation is not necessarily associated with IFN production and that different cell populations may be responsible (21, 29, 32). However, recent studies on purification of the active component in MAS suggest that the same protein moiety is responsible for both IFN production and lymphocyte proliferation (Cole et al., unpublished data). The availability of mouse strains responsive and nonresponsive to MAS with respect to IFN induction and proliferation provides a most useful model to compare the cellular requirements necessary for these activities.

Previous work by us and others suggested that intact

mycoplasmas or cell extracts were primarily inducers of IFN-α (3, 4, 11, 25–27). In fact, *M. arthritidis* organisms induced a pH- and heat-stable serum IFN as early as 6 h after injection into mice (25). The present studies indicate that *M. arthritidis* can also induce IFN-γ and that the component responsible is generated in culture supernatants. We have documented that these supernatant preparations also induce IFN-γ, but not IFN-α, in human peripheral blood mononuclear cells (13). Our previous failure to detect serum IFN-γ in mice injected with viable *M. arthritidis* could have been due to the facts that washed suspensions of organisms were used, thereby eliminating soluble products, and that the mice were not examined beyond 48 h postinjection (25). An alternative explanation is that the high titers of IFN-α may have masked low levels of IFN-γ or could have resulted in development of a hyporeactive state (10). The in vivo induction of IFN-γ by MAS and by viable organisms in various mouse strains is currently being examined.

Although it might not be considered surprising that MAS is a genetically restricted inducer of IFN-γ in view of previous proliferative data, this point needs emphasis due to the potentially important in vivo implications of the observation. IFNs are known to be potent immunoregulatory substances apart from their antiviral activities (31). Our observations suggest that microbial products may have the potential to induce IFN-γ in the naive host in a genetically restricted manner. In the case of *M. arthritidis*, which appears able to induce both IFN-α and IFN-γ, synergy between these two IFNs (2) may further accentuate the role played by the IFN-γ component and the genetic background of the host. Studies to define the significance of this synergy in vivo are currently being undertaken as are studies to evaluate the role of IFNs in chronic joint disease induced by *M. arthritidis*.

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LITERATURE CITED

1. **Aldridge, K. E., B. C. Cole, and J. R. Ward.** 1977. Mycoplasma-dependent activation of normal lymphocytes: induction of a lymphocyte-mediated cytotoxicity for allogeneic and syngeneic mouse target cells. Infect. Immun. **18**:377–385.
2. **Baron, S., J. E. Blalock, F. Dianzani, W. R. Fleishman, J. A. Georgiades, H. M. Johnson, and G. J. Stanton.** 1980. Regulatory functions of interferors. Ann. N.Y. Acad. Sci. **350**:130–143.
3. **Beck, J., H. Brunner, F. Marcucci, H. Kirchner, and J. Wietzerbin.** 1982. Induction of interferon by mycoplasmas in mouse spleen cultures. J. Interferon Res. **2**:31–36.
4. **Birke, C., H. H. Peter, V. Langenberg, W. J. P. Muller-Hermes, J. H. Peters, J. Heitmann, W. Leibold, H. Dalluge, E. Krapf, and H. Kirchner.** 1981. Mycoplasma contamination in human tumor cell lines: effect on interferon induction and susceptibility to natural killing. J. Immunol. **127**:94–98.
5. **Chanock, R. M., L. Hayflick, and M. F. Barile.** 1962. Growth on an artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Natl. Acad. Sci. U.S.A. **48**:41–49.
6. **Chen, T. R.** 1977. *In situ* detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp. Cell Res. **104**:255–262.
7. **Cole, B. C., K. E. Aldridge, and J. R. Ward.** 1977. Mycoplasma-dependent activation of normal lymphocytes: mitogenic potential of mycoplasmas for mouse lymphocytes. Infect. Immun. **18**:393–399.
8. **Cole, B. C., R. A. Daynes, and J. R. Ward.** 1981. Stimulation of mouse lymphocytes by a mitogen derived from *Mycoplasma arthritidis*. I. Transformation is associated with an H2-linked gene that maps to the IE-IC subregion. J. Immunol. **127**:1931–1936.
9. **Cole, B. C., R. A. Daynes, and J. R. Ward.** 1982. 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.
10. **Cole, B. C., J. C. Overall, Jr., P. S. Lombardi, and L. A. Glasgow.** 1975. Mycoplasma-mediated hyporeactivity to various interferon inducers. Infect. Immun. **12**:1349–1354.
11. **Cole, B. C., J. C. Overall, Jr., P. S. Lombardi, and L. A. Glasgow.** 1976. Induction of interferon in ovine and human lymphocyte cultures by mycoplasmas. Infect. Immun. **14**:88–94.
12. **Cole, B. C., G. J. Sullivan, R. A. Daynes, I. A. Sayed, and J. R. Ward.** 1982. 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.
13. **Cole, B. C., and R. N. Thorpe.** 1983. Induction of human γ interferons by a mitogen derived from *Mycoplasma arthritidis* and by phytohemagglutinin: differential inhibition with monoclonal anti-HLA/DR antibodies. J. Immunol. **131**:2392–2396.
14. **Cole, B. C., and J. R. Ward.** 1979. The mycoplasmas as arthritogenic agents, p. 367–398. *In* J. G. Tully and R. F. Whitcomb (ed.), The mycoplasmas, vol. II. Academic Press, Inc., New York.
15. **Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra.** 1979. Structural studies on the murine Ia alloantigens. III. Tryptic peptide comparisons of allelic products of the I-E/C subregion. Mol. Immunol. **16**:29–35.
16. **Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra.** 1979. Structural studies in the murine Ia alloantigens. V. Evidence that the structural gene for the I-E/C beta polypeptide is encoded within the I-A subregion. J. Exp. Med. **149**:981–986.
17. **Daynes, R. A., J. M. Novak, and B. C. Cole.** 1982. 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.
18. **Dietz, J. N., and B. C. Cole.** 1982. Direct activation of the J774.1 murine macrophage cell line by *Mycoplasma arthritidis*. Infect. Immun. **37**:811–819.
19. **Golightly-Rowland, L., B. C. Cole, J. R. Ward, and B. B. Wiley.** 1970. Effect of animal passage on arthritogenic and biological properties of *Mycoplasma arthritidis*. Infect. Immun. **1**:538–545.
20. **Green, J. A., T.-J. Yen, and J. C. Overall, Jr.** 1980. Rapid, quantitative semiautomated assay for virus-induced and immune human interferons. J. Clin. Microbiol. **12**:433–438.
21. **Guerne, P.-A., P.-F. Piguet, and P. Vassal.** 1983. Positively selected Lyt-2^+ and Lyt-2^- mouse T lymphocytes are comparable, after Con-A stimulation in release of IL2 and of lymphokines acting on B cells, macrophages, and mast cells, but differ in interferon production. J. Immunol. **130**:2225–2230.
22. **Jones, P. P., D. B. Murphy, and H. O. McDevitt.** 1978. Two-gene control of the expression of a murine Ia antigen. J. Exp. Med. **148**:925–935.
23. **Kenny, G. E.** 1967. Heat-lability and organic solvent-solubility of mycoplasma antigens. Ann. N.Y. Acad. Sci. **143**:676–681.
24. **Kenny, G. E.** 1969. Serological comparison of ten glycolytic *Mycoplasma* species. J. Bacteriol. **98**:1044–1055.
25. **Rinaldo, C. R., B. C. Cole, J. C. Overall, Jr., and L. A. Glasgow.** 1974. Induction of interferon in mice by mycoplasmas. Infect. Immun. **10**:1296–1301.

26. **Rinaldo, C. R., Jr., B. C. Cole, J. C. Overall, Jr., J. R. Ward, and L. A. Glasgow.** 1974. Induction of interferon in ovine leucocytes by species of *Mycoplasma* and *Acholeplasma*. Proc. Soc. Exp. Biol. Med. **146**:613–618.
27. **Rinaldo, C. R., Jr., J. C. Overall, Jr., B. C. Cole, and L. A. Glasgow.** 1973. Mycoplasma-associated induction of interferon in ovine leukocytes. Infect. Immun. **8**:796–803.
28. **Shreffler, D. C., and C. S. David.** 1979. P. L. Altman and D. D. Katz (ed.), Inbred and genetically defined strains of laboratory animals. I. Mouse and rat, p. 126 and 147. Federation of American Societies for Experimental Biology, Bethesda, Md.
29. **Stewart, W. E., II.** 1979. The interferon system, p. 121. Springer-Verlag, New York.
30. **Tully, J. G., and R. F. Whitcomb.** 1979. The mycoplasmas, vol. II. Academic Press, Inc., New York.
31. **Vilcek, J., I. Gresser, and T. C. Merigan.** 1980. Regulatory functions of interferons. Ann. N.Y. Acad. Sci. **350**:1–641.
32. **Wallen, W. C., J. H. Dean, and D. O. Lucas.** 1973. Interferon and the cellular immune response: separation of interferon-producing cells from DNA-synthetic cells. Cell. Immunol. **6**:110–122.
33. **Washburn, L. R., B. C. Cole, and J. R. Ward.** 1982. Chronic arthritis of rabbits induced by mycoplasmas. III. Induction with non-viable *Mycoplasma arthritidis* antigens. Arthritis Rheum. **25**:937–946.
34. **Wiranowska-Stewart, M., and W. E. Stewart.** 1981. Determination of human leukocyte populations involved in production of interferons alpha and gamma. J. Interferon Res. **1**:233–244.
35. **Yowell, R. L., B. C. Cole, and R. A. Daynes.** 1983. Utilization of T-cell hybridomas to establish that a soluble factor derived from *Mycoplasma arthritidis* is truly a genetically-restricted polyclonal T-cell mitogen. J. Immunol. **131**:543–545.