INFECTION AND IMMUNITY, Sept. 1989, p. 2804–2810
0019-9567/89/092804-07$02.00/0

Vol. 57, No. 9

Parasite Exposure Elicits a Preferential T-Cell Response Involved in Protective Immunity against *Eimeria* Species in Chickens Primed by an Internal-Image Anti-Idiotypic Antibody

BALBIR S. BHOGAL,†* ETHEL B. JACOBSON, HARLEY Y. TSE,‡ DENNIS M. SCHMATZ, AND OWEN J. RAVINO

Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received 24 April 1989/Accepted 16 June 1989

Polyclonal anti-idiotype 1073 (anti-Id 1073), raised against a monoclonal antibody specific for the protective epitope(s) of *Eimeria tenella* sporozoites, induced cell-mediated immune (CMI) responses in bursectomized chickens. Whereas alhydrogel-adsorbed anti-Id 1073 was sufficient to engender the CMI response at 4 h after injection, induction of the CMI response at 24 h required both alhydrogel and muramyl dipeptide sterol. Exposure of immunized chickens to live parasites prompted a dichotomous effect on the CMI response engendered by anti-Id in that the 4-h CMI response was preferentially stimulated and the 24-h CMI response was down regulated. Both types of CMI response were transferable to naive chickens by T cells from anti-Id 1073 immune donors or by parasite-specific T cells from clones 21 and 27. These T-cell clones were generated from chickens immunized by repeated infections with *E. tenella* and showed in vitro proliferative responses to anti-Id 1073. The abilities of T cells from clone 21 to selectively transfer the 4-h CMI response and to generate gamma interferon to activate macrophages for their cytotoxic effects on *Eimeria* sporozoites correlate with the preferential stimulation by parasites of the 4-h CMI response in chickens immunized with anti-Id 1073. These data show that anti-Id 1073 mimics the protective epitope(s) of the parasite and primes chickens for protective CMI responses. Cytotoxic T cells, equivalent to the mammalian T-cell subset of the Lyt2$^+$ phenotype, appear to be the primary effector T cells in the CMI response engendered by anti-Id 1073 against *Eimeria* parasites.

The immune network hypothesis (22) predicts that anti-idiotypic antibodies (anti-Id; Ab-2), in particular the Ab-2β type (23), produced against the paratope-associated idiotypic determinants (Id) of a given antibody molecule (Ab-1) may represent an internal image of the antigen recognized by Ab-1. This concept has been validated by much experimental data (1, 11–13, 15, 28, 29, 45, 49, 50, 52, 54), and recent studies on successful application of anti-Id as vaccines provide further support for this concept (5, 17, 25, 28, 29, 32, 41, 45). Experimentally, it has been shown that anti-Id directed against B-cell Id can activate functionally distinct subsets of T cells, such as those that mediate a delayed-type hypersensitivity (DTH) response (50, 52), T suppressor cells (49) in mice, and T helper cells that regulate B-cell responses in mice (11) and in chickens (5).

We have generated a number of polyclonal anti-Id, using *Eimeria tenella* sporozoite-specific monoclonal antibodies, and one of them (anti-Id 1073), when used as a vaccine, has been shown to induce specific antibody responses and protective immunity against the parasite infection in chickens (5). Since T cells play a critical role in protective immunity against *Eimeria* parasites (6, 16, 31, 39), the ability of anti-Id to induce cell-mediated immune (CMI) responses in chickens was studied. Anti-Id functions as a unique surrogate of protective antigen, and bursectomized (BX) chickens allow a more critical study of T-cell function than the murine system and also provide a biologically relevant model for coccidiosis caused by protozoan parasites of the genus *Eimeria*.

The economic importance of the disease has fostered an outburst of interest in the development of a vaccine, and the feasibility of a molecular vaccine for coccidiosis has been indicated by recent studies (3, 33). Anti-Id, with its potential as a surrogate antigen, may provide a better alternative for vaccinating 1-day-old outbred broiler chickens against coccidiosis, since anti-Id appears to override the problems associated with the immaturity of the neonate immune system (15, 18, 45) and those caused by genetic restriction in generating T-cell responses (12, 13, 25). The results presented here show that anti-Id 1073 induced protective CMI responses in BX chickens and substantiate our earlier observations showing that it could prime T cells in chickens with intact bursas to engender parasite-specific B-cell responses upon exposure to parasites (5).

MATERIALS AND METHODS

Chickens. Two-week-old White Leghorn chickens (SC, B2/B2) and 1-day-old outbred broiler chickens were used in this study. SC chickens were purchased as fertilized eggs from Hy-Line International, Dallas Center, Iowa. Eggs were incubated and hatched in a model 5 incubator-hatcher (Petersime Incubator Co., Gettysburg, Ohio). Outbred (Hubbard/Hubbard) broiler chickens were purchased as day-old birds from a local supplier (Kerr Hatcheries, Frenchtown, N.J.). Chickens were kept in clean coccidium-free rooms during the period of experimentation.

Bursectomy. SC chickens were BX by injecting 3.75 mg of testosterone propionate (U.S. Biochemical Corp., Cleveland, Ohio) on day 11 of embryonation, followed by an injection of cyclophosphamide (Cytoxan; Meade Johnson, Evansville, Ind.) after hatching (4 mg on day 1 and 3 mg on

* Corresponding author.
† Present address: Department of Molecular Biology, A. H. Robins Research Laboratories, 1211 Sherwood Avenue, Richmond VA 23261-6609.
‡ Present address: Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI 48201.

day 2, both intraperitoneally). Chickens were tested for the absence of immunoglobulins in their sera from the age of 3 weeks to the end of the experiment by immunodiffusion and enzyme-linked immunosorbent assay. Only agammaglobulinemic chickens were included in the results.

Parasites and antigens. Oocysts and sporozoites from *E. tenella* or other *Eimeria* species were prepared as described elsewhere (43). Sporulated oocyst antigen (SOA) and sporozoite antigen (Sp Ag) were prepared by sonication of oocysts or sporozoites as described previously (5).

Preparation, characterization, and specificity of anti-Id. Anti-Id, prepared against the Id of a monoclonal antibody (antibody 1073) with specificity for the sporozoites of *E. tenella*, were raised in rabbits and characterized as described previously (5).

Immunizations of chickens. SC normal (SCNL) and SCBX chickens were immunized intramuscularly at the age of 14 days by injecting 50 μg of anti-Id or *E. tenella* Sp Ag, using alhydrogel (30%, vol/vol) alone or in combination with 100 μg of *N-O*-acetylmuramyl-alanyl-D-glutamine-6-*O*-stearoyl (MDPS; Calbiochem-Behring, La Jolla, Calif.). Chickens were given booster injections twice of 200 μg of anti-Id or *E. tenella* Sp Ag, using alhydrogel or both alhydrogel and MDPS (days 21 and 28). One-day-old broiler chickens were similarly immunized with alhydrogel-adsorbed *E. tenella* Sp Ag or anti-Id on days 1, 14, and 21 with and without MDPS.

Assays of CMI responses. CMI responses in chickens immunized with anti-Id or *E. tenella* Sp Ag were assayed by DTH reaction in the wattle (WDTH). Fifteen days after the last immunization, the left wattles of chickens (six per group) were injected intradermally with 50 μg of *E. tenella* SOA. The right wattles were injected either with 50 μg of bovine serum albumin or with 50 μg of a heterologous *Eimeria* antigen, as specified in Results. The wattle thicknesses were measured at various time intervals with a micrometer (Schnelltaster System Kroplan, Federal Republic of Germany). The magnitude of the WDTH response was expressed as mean increases in thickness in the wattles challenged with *E. tenella* SOA compared with the control wattles. To study the effect of challenge infection on WDTH responses, anti-Id-immunized chickens were infected with 50,000 sporulated oocysts of *E. tenella* 15 days after the last immunization. Each day after infection, three chickens were challenged with *E. tenella* SOA in the left wattles and with bovine serum albumin in the right wattles. The WDTH responses were measured at 4 and 24 h. Statistical evaluation of data was done by using Dunnett's one-tailed test.

Assay of lesion scores. Six days after infection, as described above for the WDTH assays, 9 to 14 chickens from various groups were sacrificed by CO_2 asphyxiation, and lesions in their ceca were scored on a scale of 0 (no damage to the cecal mucosal tissue) to 4 (maximum damage to the cecal tissue), as described elsewhere (24).

Establishment of chicken T-cell clones. Parasite-specific T-cell clones were established from chickens immunized by repeated infections with *E. tenella* and were maintained as described previously (6). These clones were characterized for the specificity for parasite antigens, in vitro functions (6), the ability to produce interleukin-2 and gamma interferon (IFN-γ), and interactions with anti-Id (B. S. Bhogal, E. B. Jacobson, and D. M. Schmatz, manuscript in preparation). Clones 21 and 27 were of special interest, since T cells from these clones were stimulated by anti-Id 1073 in vitro.

Adoptive transfer of CMI responses. Spleens from SCBX chickens immunized with anti-Id or *E. tenella* Sp Ag were removed 10 days after the last immunization, and single-cell suspensions were prepared in 1.1× Dulbecco phosphate-buffered saline, pH 7.3. Cells were washed three times, counted, and suspended in 1.1× phosphate-buffered saline containing 5% fetal calf serum. T cells in the splenic lymphocyte preparations were depleted by anti-T-cell antibody and complement treatment. Briefly, spleen cells (10^7/ml) were incubated with a rabbit anti-chicken T-cell serum (kindly supplied by G. J. Thorbecke, New York University Medical Center, New York, N.Y.) for 30 min on ice. Guinea pig complement was then added to the cells in a final dilution of 1:18, and the cells were further incubated at 37°C for 1 h. The cells were washed with phosphate-buffered saline three times, resuspended, and counted. Spleen cells (5×10^7) or parasite-specific T cells from clone 21 or 27 (1×10^6) were transferred intravenously into 4-week-old naive SCNL chickens. A group of chickens received 10 ml of serum obtained from one of the spleen cell donor chickens, and another group received serum from immunized SCNL chickens. Eighteen hours after the transfer of cells, chickens were challenged with 50 μg of *E. tenella* SOA in their left wattles and with control antigens in their right wattles, and WDTH responses were measured at 4 and 24 h later.

RESULTS

CMI responses in immunized chickens. Immunization of SCBX, SCNL, or broiler chickens with polyclonal anti-Id 1073 or *E. tenella* Sp Ag with alhydrogel and MDPS as adjuvants induced specific CMI responses to intradermally injected *E. tenella* SOA, as measured by WDTH (Fig. 1). In view of its superior ability to induce DTH in chickens (38), MDPS was used to induce the CMI response with anti-Id. The CMI responses followed kinetics similar to those of the typical murine DTH (44, 50), in that the peak CMI responses were observed at 24 h after injection of parasite antigen (24-h CMI response), with some swelling in the wattles as early as 4 h after injection (4-h CMI response). The histological observations were also characteristic of the murine DTH (data not shown). The CMI response levels came down by 48 to 72 h, as evidenced by decreases in the wattle thicknesses, and at 72 h the thicknesses in the wattles were only marginally greater than those of the control wattles. The 24-h CMI response levels in SCBX chickens (Fig. 1A) were lower than those observed in the SCNL and broiler chickens (Fig. 1B, data pooled for SCNL and broilers). In general, SCNL, SCBX, and broiler chickens immunized with anti-Id showed lower 24-h CMI response values than did those immunized with *E. tenella* Sp Ag, but the kinetics of the responses induced by the two antigens were comparable. The levels of the 24-h CMI responses elicited by alhydrogel-adsorbed anti-Id or *E. tenella* Sp Ag were lower than those observed when both adjuvants (alhydrogel and MDPS) were used; however, these differences were not reflected in the 4-h CMI responses.

Specificity of the CMI responses. Chickens immunized with *E. tenella* Sp Ag, using MDPS and alhydrogel, showed 24-h CMI responses when challenged with SOA prepared from the heterologous strains of *E. tenella*, but the CMI response values were much lower than those observed with the SOA or schizont antigen from the homologous strain (LS18; Table 1). The values for the 24-h CMI responses to the heterologous *Eimeria* species, i.e., *E. acervulina* and *E. maxima*, were also considerably lower (wattle thickness increases of 0.38 and 0.40 mm, respectively) than those induced by *E. tenella* LS18 antigens. Interestingly, the CMI response levels of the chickens immunized with anti-Id to heterologous

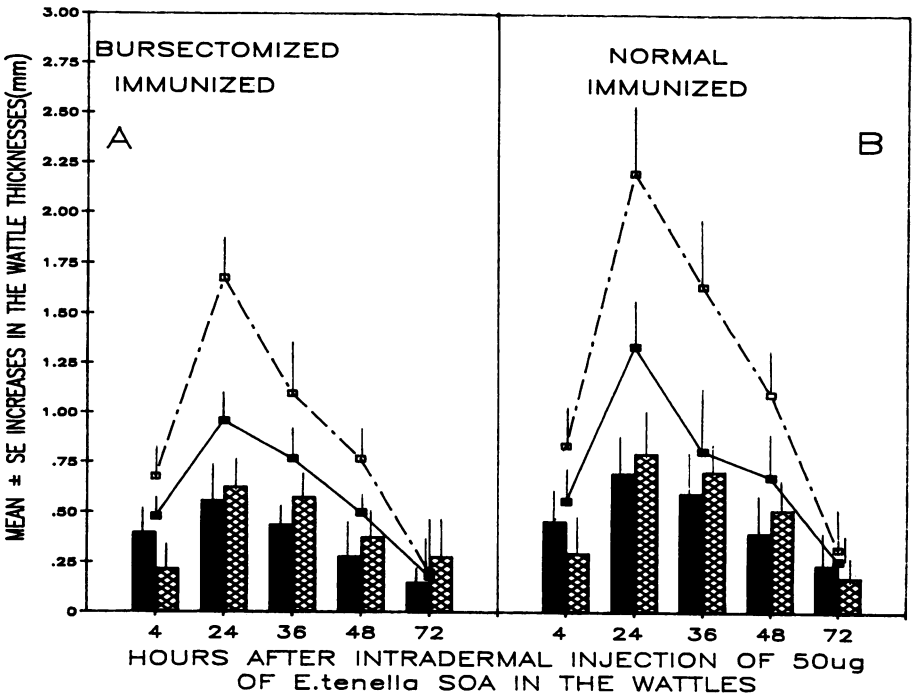

FIG. 1. CMI responses to parasite antigens in BX (A) and normal (B) chickens immunized with anti-Id 1073, using either alhydrogel (▮) or alhydrogel plus MDPS (■——■) and in chickens immunized with *E. tenella* Sp Ag, using either alhydrogel (▩) or alhydrogel plus MDPS (□— - —□). CMI responses were measured as swelling in the wattles and are shown as the mean ± standard error of increases in wattle thicknesses for 6 (A) or 12 (B) chickens at each datum point.

parasite antigens were significantly higher than those observed in chickens immunized with parasite antigens. The higher levels of CMI response engendered by vaccination with anti-Id against the heterologous parasite antigens may simply reflect the relative quantitative preponderance of the relevant CMI response-inducing epitope(s) in the anti-Id preparations, compared with that in the crude parasite antigen preparations. In the absence of purified parasite antigens, use of anti-Id clearly indicates the presence of some T-cell antigens cross-reactive between *Eimeria* strains, as well as between the *Eimeria* species tested.

Adoptive transfer of CMI responses. To define the cellular nature of the CMI responses to parasite antigens, adoptive transfer studies were performed. Spleen cells from SCBX chickens immunized with anti-Id 1073 or *E. tenella* Sp Ag transferred CMI responses to *E. tenella* antigens in the naive SCNL recipient birds (Fig. 2). The immune spleen cells responsible for adoptive transfer of the CMI response were T cells, since treatment of spleen cells with anti-T-cell antibody and complement before transfer abrogated the ability of these cells to transfer CMI responses. Although the spleen cell donor SCBX chickens showed no immunoglobulin in the sera by immunodiffusion assays, the confirmation of their agammaglobulinemia by a more sensitive assay, such as enzyme-linked immunosorbent assay, was not done until after the end of the study. Anti-T-antibody treatment of splenocytes was therefore done to override the uncertainty of the BX status of the donors. Parasite-specific cloned T cells from clones 21 and 27 also transferred CMI responses. Chickens which received T cells (10^6) from clone 27 preferentially manifested a high level of 24-h CMI responses, whereas those which received T cells (10^6) from clone 21 failed to show a high level of the 24-h CMI response but mounted the 4-h CMI response. A combination of T cells (2×10^6) from both these clones, when injected intravenously or in the wattles along with antigen, transferred both of the responses. Immune sera from immune SCBX or SCNL chickens failed to transfer CMI responses (data not shown),

TABLE 1. Specificities of CMI responses induced in chickens by anti-Id

Immunization treatment[a]	Mean ± SE increase in wattle thickness (mm) 24 h after injection of antigen[b]						
	E. tenella					*E. acervulina*	*E. maxima*
	LS18 (SOA)	LS18 (Sch. Ag)	DP84 (SOA)	DP72 (SOA)	FS13 (SOA)	LS3 (SOA)	FS110 (Sch. Ag)
Anti-Id 1073	1.28 ± 0.45	1.76 ± 0.34	0.67 ± 0.21*[c]	0.58 ± 0.16*	0.73 ± 0.22*	0.68 ± 0.27*	0.83 ± 0.31*
E. tenella LS18 Sp Ag	1.69 ± 0.28	1.36 ± 0.30	0.31 ± 0.10**	0.24 ± 0.09**	0.38 ± 0.09**	0.33 ± 0.11**	0.40 ± 0.08**

[a] BX chickens were immunized with anti-Id 1073 or *E. tenella* Sp Ag, using both alhydrogel and MDPS as adjuvants.

[b] Antigen (50 μg) was injected intradermally. The mean increase ± SE in the thicknesses of wattles injected with 50 μg of bovine serum albumin as a control antigen was 0.18 ± 0.06 mm. Sch. Ag, Second-generation schizont antigen of the parasite. Strain LS18 was used for producing monoclonal antibody against which anti-Id was generated. DP84, DP72, and FS13 are field isolates of *E. tenella*. LS3 and FS110 are laboratory strains of *E. acervulina* and *E. maxima*, respectively.

[c] * and **, Significantly different ($P \leq 0.05$) CMI responses; comparisons are made within columns.

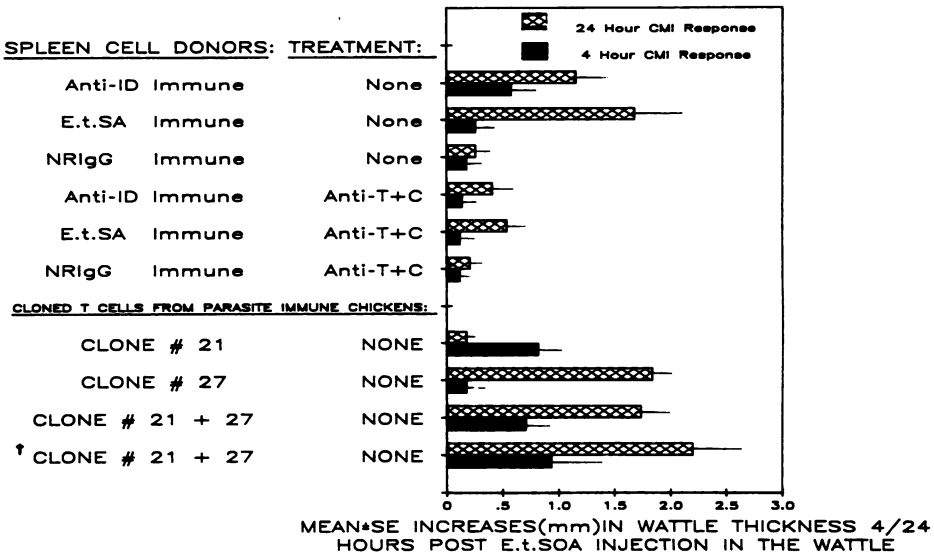

FIG. 2. Adoptive transfer of 4- and 24-h CMI responses in chickens by splenic T lymphocytes (5×10^7) from BX donor chickens immunized with anti-Id 1073 or *E. tenella* Sp Ag or by T cells from clone 21 (1×10^6 cells) or 27 (1×10^6 cells), injected intravenously. Transfer of CMI response by a combination of T cells from both clones (2×10^6 cells), injected intravenously (clone # 21 + 27) or locally in the wattle (†clone # 21 + 27), is also shown. Each datum point represents a mean for three to six chickens. E.t., *E. tenella*.

thus suggesting that the responses were not transferable by soluble factors.

Comparable abilities of anti-Id and *E. tenella* Sp Ag to induce protective immunity in inbred and outbred chickens. Chickens immunized with anti-Id 1073 in the presence of alhydrogel and MDPS showed resistance to an experimental *E. tenella* infection, as evidenced by reduced cecal lesion scores observed in the immunized chickens. The virulence of the parasites and the susceptibility of chickens to infection were evident from the high lesion scores observed in the control chickens, which received vehicle only (Table 2). The degree of protective immunity in SCBX, SCNL, and broiler chickens induced by anti-Id immunization was comparable to, if not higher than, that induced by the parasite antigen, since the mean lesion score in the groups of chickens immunized with anti-Id 1073 and those immunized with *E. tenella* Sp Ag do not show statistical differences ($P < 0.01$). Interestingly, however, the evaluation of these data on the basis of the distribution of chickens in various ranges of lesion scores suggests that vaccination with anti-Id was more efficacious than the vaccination with the parasite antigens, since 10 of 14 (71%) of the inbred chickens vaccinated with anti-Id had lesion scores in the range of 0 to 1, compared with 6 of 13 (46%) of the *E. tenella* Sp Ag vaccinates. A similar but less striking effect was also observed in the outbred chickens, of which 8 of 14 (57%) chickens vaccinated with anti-Id had lesion scores of 0 to 1, compared with 6 of 14 (43%) Sp Ag vaccinates.

TABLE 2. Comparative abilities of anti-Id and parasite antigen to vaccinate chickens against *E. tenella* infection[a]

Immunization treatment	Chicken (*n*)	No. of chickens with lesion score in the range of:				Lesion score (mean ± SE)[b]
		0–1.0	1.2–2.0	2.2–2.7	3.0–4.0	
Anti-Id 1073	SCNL (14)	10	3	1	0	1.04 ± 0.19*
	SCBX (12)	6	3	2	1	1.63 ± 0.30*
	Broiler (14)	8	4	1	1	1.29 ± 0.22*
Sp Ag	SCNL (13)	6	5	2	0	1.65 ± 0.21*
	SCBX (11)	4	5	1	1	1.75 ± 0.19*
	Broiler (14)	6	4	3	1	1.86 ± 0.25*
Vehicle only	SCNL (10)	0	1	2	7	3.55 ± 0.24**
	SCBX (9)	0	1	1	7	3.08 ± 0.28**
	Broiler (12)	0	0	2	10	3.50 ± 0.18**

[a] SCNL and SCBX chickens were immunized at 14, 21, and 28 days of age, using both alhydrogel and MDPS as adjuvants. Broiler chickens were immunized on days 1, 14, and 21, using both adjuvants. Cecal lesions were scored 6 days postinfection with *E. tenella* oocysts. The challenge inoculum of oocysts was pretitrated to obtain lesion scores in the range of 3 to 4 in the unimmunized susceptible control chickens.

[b] * and **, Significantly different ($P \leq 0.05$) mean lesion scores.

Dichotomous effects of infection on CMI responses. In a preliminary experiment, it was observed that chickens immunized with anti-Id 1073 showed decreased 24-h CMI response levels on day 6 postinfection, compared with the CMI responses observed before infection. To study the kinetics of the depression in specific CMI responses, SCBX chickens immunized with anti-Id were infected with *E. tenella* 15 days after the last immunization, and each day postinfection, groups of three chickens were assayed for CMI responses. A clear depression in the 24-h CMI response levels was observed in the chickens immunized with anti-Id (Fig. 3), with maximal depression on days 4 to 10 postinfection. By day 15 postinfection, the 24-h CMI responses returned to levels similar to those observed before infection. In contrast, after exposure to the parasite, the 4-h CMI responses were increased for the first 72 h postinfection, being maximal at 24 to 36 h.

DISCUSSION

The present data show that immunization with anti-Id 1073 induced *Eimeria* sp.-specific CMI responses in chickens which had no previous exposure to the parasite. The moderate CMI responses, in particular the 24-h CMI responses induced by vaccination with alhydrogel-adsorbed anti-Id

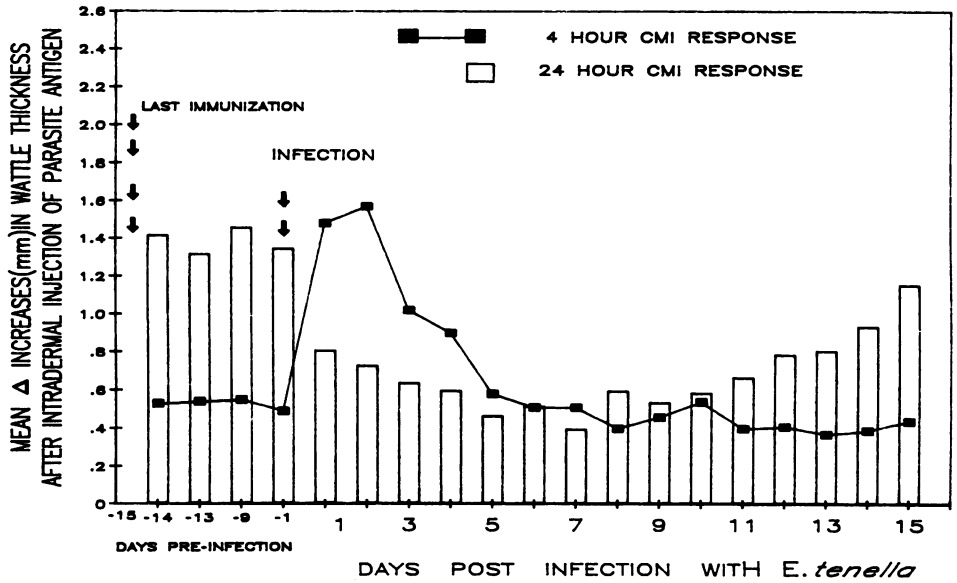

FIG. 3. Dichotomous effects of exposure to parasites on the 4- and 24-h CMI responses in BX chickens immunized with anti-Id 1073. Fifteen days after the last immunization, chickens were infected with 50,000 *E. tenella* oocysts, and three chickens were assayed for CMI responses every day postinfection.

1073, were increased in potency when the anti-Id 1073 was injected in the presence of alhydrogel and MDPS. Both the 4-h and the 24-h CMI responses were mediated by T cells, since these responses were transferable by splenic lymphocytes from SCBX chickens immunized with anti-Id or by parasite-specific cloned T cells. BX chickens lack antibodies, and since the potencies of the 4-h responses in SCBX and SCNL chickens were comparable, these responses were not due to immune complexes. Several studies performed in the murine system suggest that T cells bear Id-like determinants recognizable by anti-Id produced against Id expressed on immunoglobulin (1, 11, 44, 49, 50, 52). The results presented here corroborate those observations, using a more stringent model (BX chickens) than the murine system to study T-cell activation by anti-Id.

It is well established that the CMI response plays a critical role in resistance against *Eimeria* infections, since (i) BX chickens, which lack the B-cell component of their immune system, can be protected (6, 31), and (ii) macrophages (Mϕ) activated by IFN-γ secreted by parasite-specific cloned T cells can effectively kill sporozoites in vitro (6). It is, however, not clear which subset of T cells is most important in the protective CMI responses. The mechanism of induction of CMI responses by anti-Id is also not known, and both of these parameters are critical in the design of a vaccine capable of stimulating only the protective T-cell responses.

The CMI responses in immunized chickens may have been induced via recognition by T cells of anti-Id as a surrogate of the nominal parasite antigen in association with the B-L/B-F (murine equivalents of class II and class I molecules, respectively) gene products on the surface of histocompatible antigen-presenting cells. Alternatively, anti-Id may bypass the B-L/B-F restriction to stimulate T cells by virtue of its high affinity for at least the antigen-binding portion of the T-cell receptor, as has been shown in studies on immunization of mice with anti-Id against Sendai virus (12, 13) and against *Listeria monocytogenes* (25). In this context, it is interesting that anti-Id was relatively more effective in inducing protective immunity in broiler chickens than were the parasite antigens. Furthermore, anti-Id 1073 stimulated T cells from two clones with distinct in vitro functional activities. T cells from clone 27, which showed in vitro helper activity for B cells (6), required histocompatible antigen-presenting cells for anti-Id-induced in vitro proliferation, produced T-cell growth factor containing putative interleukin-2 (Bhogal et al., manuscript in preparation), and transferred 24-h CMI responses (Fig. 2). T cells from clone 21, which produced IFN-γ and transferred 4-h CMI responses (Fig. 2), showed no such requirement for anti-Id-induced in vitro proliferation, even though they required histocompatible antigen-presenting cells for similar responses to parasite antigens (Bhogal et al., manuscript in preparation). Since the T cells were isolated from parasite-immune chickens and cloned after a relatively short period of in vitro stimulation with the parasite antigen without an extended selection process in the absence of nonspecific mitogens, the in vitro activities of these T-cell clones may be representative of the in vivo scenario in an immune chicken. The relevance of the in vitro functional activities of these T-cell clones in protective immunity is borne out by the dichotomous effects of parasite infection on the CMI responses in chickens immunized with anti-Id (Fig. 3), in that the 4-h CMI responses were preferentially restimulated, whereas the 24-h responses were down regulated. It will be of interest to examine whether a similar dichotomous response is also generated in parasite antigen-immune chickens. However, because of the lack of a purified antigen, we used anti-Id as a prototype of a protective epitope of the parasite to elucidate the relative roles of different T-cell subsets in protective immunity. Unfortunately, because of a lack of reagents with which to identify phenotypic markers on subsets of T cells in chickens, the phenotypic definition of the putative T-cell subset(s) involved in this response is not possible at present.

Studies using cloned T cells in mice suggest a synergistic interaction between the $Lyt2^+$ (cytotoxic) and $L3T4^+$ (helper) T cells in protective immunity against various pathogens (10, 27, 47, 48). Nonetheless, it is clear from studies done with cloned T cells that the Th-1 type $L3T4^+$ T-cell subset transfers the typical (24-h) DTH response and that the Th-2 type $L3T4^+$ and the $Lyt2^+$ subsets do not (9, 19, 34).

The primed Lyt2$^+$ T cells, in addition to their activity as cytotoxic T cells, secrete antigen-stimulated IFN-γ, a potent lymphokine capable of activating Mϕ for enhanced killing of both intracellular and extracellular pathogens (10, 26, 35–37). In view of the background described above and (i) a preferential stimulation of the 4-h CMI response by parasite exposure in anti-Id-immunized chickens and (ii) the selective ability of two functionally distinct cloned T cells to transfer 24-h and 4-h CMI responses, the protective immunity in the chickens appears to be mediated by a population of Lyt2$^+$-like T cells. The putative Lyt2$^+$-like cytotoxic T cells, when driven to expand by the antigenic epitope(s), on exposure to live parasites secreted IFN-γ or other factors to lyse the host cells carrying the parasites, while at the same time activating Mϕ. Activated Mϕ may cause parasite attrition outside the host cells or, alternatively, in the host cells, as has been shown for malarial parasites (14). Consistent with this observation, studies with other parasites have shown that a nonliving vaccine fails to generate protective immunity against *Schistosoma mansoni* in certain strains of mice, even when Th-1 type L3T4$^+$ cell functions, i.e., ability to mount the DTH and antibody responses, are intact (20, 21). The failure of these mice to mount a protective immune response is attributed to their inability to generate Mϕ-activating factors. Likewise, the development of resistance to *Leishmania major* in genetically susceptible mice has been shown to require depletion of an L3T4$^+$ T-cell subset (42, 53). In a recent study, L3T4$^+$ cells have been shown to play a predominant role in protective immunity against *Eimeria vermiformis* in mice (40); however, since the study was not performed with cloned T cells, small numbers of contaminating Lyt2$^+$ T cells may have contributed to the observed protective effects, especially when large numbers of L3T4$^+$ cells (2×10^8 to 3×10^8 cells per mouse) were transferred. It may be argued that the Th-2 type L3T4$^+$ T-cell subset that produces IFN-γ may have been involved in protection, but the class II-restricted cytotoxic activity of L3T4$^+$ or CD4$^+$ helper-inducer T cells is generally seen in permanent T-cell lines and is rare or absent in the original starting cultures or circulating T cells (7). The involvement of other effector cells, such as the NK cells or even polymorphonuclear leukocytes, which are also activated by IFN-γ (46) and have been shown to be important effector cells of the CMI response in the intestinal tract (4), needs careful investigation.

Nonspecific immunodepression has been observed in parasitic infections (30), but the specific aspects of it are not clear and neither is its significance in regulation of parasite-specific responses. In general, the 24-h CMI response is difficult to induce in chickens without using potent adjuvants (38), and, interestingly, in the present study the 24-h CMI response did not correlate with protection. Such a response may therefore represent a bystander effect caused by activation of the Th-1 type L3T4$^+$ subset of T cells, which may be involved in the early regulatory phases of the CMI response, as has been recently shown in studies on the generation of CMI responses to *Listeria monocytogenes* (2) and to *Trypanosoma cruzi* (51) in mice. In the light of growth and functional properties of mammalian Th-1 and Th-2 type L3T4 cells (9, 34), it is possible that the T-cell subset (represented by clone 21) stimulated more strongly during priming with anti-Id encourages similar responses upon exposure to parasites and inhibits the other subset (clone 27) on a reciprocal basis. In conclusion, protective immunity against *Eimeria* parasites in chickens appears to be mediated by T cells similar to those that induce the 4-h CMI response, which may be represented by T cells from clone 21, which display the properties of a cytotoxic T cell. With the establishment of technology for cloning parasite-specific T cells in chickens (6) and development of monoclonal antibodies for identification of some of the marker molecules, such as CD4 and CD8 (8), similar to those of mammals, a clearer definition of T cells in chickens is foreseeable.

ACKNOWLEDGMENTS

We are most grateful to G. J. Thorbecke, New York University Medical Center, New York, N.Y., for a generous supply of anti-T-cell antibody and for inbred chickens and to Kitty Burton, Department of Molecular Biology, A. H. Robins Research, for preparation of the manuscript.

LITERATURE CITED

1. **Arnold, B., R. Wallich, and G. U. Haemmerling.** 1982. Elicitation of delayed type hypersensitivity response to phosphorocholine by monoclonal anti-idiotypic antibodies in an allogeneic environment. J. Exp. Med. **156**:670–674.
2. **Berche, P., C. Decreusefond, I. Theodorou, and C. Stiffel.** 1989. Impact of genetically regulated T cell proliferation on acquired resistance to Listeria monocytogenes. J. Immunol. **132**:932–939.
3. **Bhogal, B. S., G. A. Miller, A. C. Anderson, E. J. Jessee, R. Strausberg, and S. Strausberg.** 1989. Vaccination of chickens with recombinant *E. tenella* antigen alone or in combination with a subclinical exposure induces cross protective immunity against coccidiosis. Prog. Clin. Biol. Res. **307**:131–146.
4. **Bhogal, B. S., L. K. Nagy, and P. D. Walker.** 1987. Neutrophil mediated and IgA dependent antibacterial immunity against enteropathogenic *Escherichia coli* in the porcine intestinal mucosa. Vet. Immunol. Immunopathol. **14**:23–44.
5. **Bhogal, B. S., K. H. Nollstadt, Y. D. Karkhanis, D. M. Schmatz, and E. B. Jacobson.** 1988. Anti-idiotypic antibody with potential use as an *Eimeria tenella* sporozoite antigen surrogate for vaccination of chickens against coccidiosis. Infect. Immun. **56**:1113–1119.
6. **Bhogal, B. S., H. Y. Tse, E. B. Jacobson, and D. M. Schmatz.** 1986. Chicken T lymphocyte clones with specificity for *Eimeria tenella*: generation and functional characterization. J. Immunol. **137**:3318–3325.
7. **Bourgault, I., A. Gomez, E. Gomrad, F. Picard, and J. P. Levy.** 1989. A virus-specific CD4 cell-mediated cytotoxic activity revealed by CD8 cell elimination regularly develops in uncloned human antiviral cell lines. J. Immunol. **142**:252–256.
8. **Chan, M. M., C.-L. H. Chen, L. L. Ager, and M. D. Cooper.** 1986. Identification of the avian homologues of mammalian CD4 and CD8 antigens. J. Immunol. **140**:2133–2138.
9. **Cher, D. J., and T. R. Mossmann.** 1987. Two types of murine helper T cell clones. II. Delayed-type hypersensitivity is mediated by Th1 clones. J. Immunol. **138**:3688–3694.
10. **Chiplunkar, S., G. De Libero, and S. H. E. Kaufmann.** 1986. *Mycobacterium leprae*-specific Lyt-2$^+$ T lymphocytes with cytolytic activity. Infect. Immun. **54**:793–797.
11. **Eichmann, K., and K. Rajewsky.** 1975. Induction of T and B cell immunity by anti-idiotypic antibody. Eur. J. Immunol. **5**:661–668.
12. **Ertl, H. C. J., and R. W. Finberg.** 1984. Sendai virus specific T cell clones: induction of cytolytic T cell clones by anti-idiotypic antibody directed against a helper T cell clone. Proc. Natl. Acad. Sci. USA **81**:2850–2854.
13. **Ertl, H. C. J., E. Homans, S. Thomas, and R. W. Finberg.** 1984. Sendai virus specific T cell clones. V. Induction of a DTH response using an anti-idiotypic antibody. J. Exp. Med. **81**: 1720–1727.
14. **Ferreira, A., L. Schofield, V. Enea, H. Schellekens, P. van der Meide, W. E. Collins, R. S. Nussenzweig, and V. Nussenzweig.** 1986. Inhibition of development of exoerythrocytic forms of malaria parasite by gamma-interferon. Science **232**:881–884.
15. **Francotte, M., and J. Urbain.** 1984. Induction of anti-tobacco mosaic virus antibodies in mice by rabbit anti-idiotypic antibod-

ies. J. Exp. Med. **160**:1485–1494.
16. **Giambrone, J. J., P. H. Klesius, and S. A. Edgar.** 1980. Avian coccidiosis: evidence for a cell mediated immune response. Poult. Sci. **59**:38–43.
17. **Grzych, J. M., M. Capron, P. H. Lambert, C. Dissous, S. Torres, and A. Capron.** 1985. An anti-idiotype vaccine against experimental schistosomiasis. Nature (London) **316**:74–76.
18. **Hiernaux, J., C. Bona, and P. J. Baker.** 1981. Neonatal treatment with low doses of anti-idiotypic antibody leads to the expression of a silent clone. J. Exp. Med. **153**:1004–1008.
19. **Hussein, S., J. Curtis, H. Akuffo, and J. L. Turk.** 1987. Dissociation between delayed-type hypersensitivity and resistance to pathogenic mycobacteria demonstrated by T-cell clones. Infect. Immun. **55**:564–567.
20. **James, S. L., and L. A. DeBlois.** 1986. Induction of protective immunity against *Schistosoma mansoni* by a non-living vaccine. II. Response of mouse strains with selective immune defects. J. Immunol. **136**:3864–3871.
21. **James, S. L., C. Salzman, and E. J. Pearce.** 1988. Induction of protective immunity against *Schistosoma mansoni* by a non-living vaccine. VI. Antigen recognition by non-responder mouse strains. Parasite Immunol. **10**:71–83.
22. **Jerne, N. K.** 1974. Towards a network theory of the immune system. Ann. Immunol. (Paris) **124c**:373–389.
23. **Jerne, N. K., J. Roland, and P. A. Cazenave.** 1982. Recurrent idiotypes and internal images. EMBO J. **1**:243–247.
24. **Johnson, J., and W. M. Reid.** 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Exp. Parasitol. **28**:30–38.
25. **Kaufmann, S. H. E., K. Eichmann, I. Müller, and L. J. Wrazel.** 1985. Vaccination against the intracellular bacterium *Listeria monocytogenes* with a clonotypic antiserum. J. Immunol. **134**: 4123–4127.
26. **Kaufmann, S. H. E., H. Hahn, R. Beger, and H. Kirchner.** 1983. Interferon-gamma production by *Listeria monocytogenes* specific T cells active in cellular immunity. Eur. J. Immunol. **13**:265–268.
27. **Kaufmann, S. H. E., E. Hug, U. Väth, and I. Müller.** 1985. Effective protection against *Listeria monocytogenes* and delayed-type hypersensitivity to listerial antigens depend on cooperation between specific L3T4$^+$ and Lyt 2$^+$ T cells. Infect. Immun. **48**:263–266.
28. **Kennedy, R. C., K. Adler-Torthz, R. D. Hemkel, Y. Sanchez, J. L. Melnick, and G. R. Dreesman.** 1983. Immune response to hepatitis B surface antigen: enhancement by prior injection of antibodies to the idiotype. Science **221**:853–855.
29. **Kennedy, R. C., and G. R. Dreesman.** 1984. Enhancement of the immune response to hepatitis B surface antigen: *in vivo* administration of anti-idiotype induces anti-HBs that express a similar idiotype. J. Exp. Med. **159**:655–665.
30. **Krettli, A. W., and F. E. L. Pereira.** 1981. Immunosuppression in protozoal infections, p. 449–462. *In* M. Levankowsky and S. H. Hunter (ed.), Biochemistry and physiology of protozoa, vol. 4. Academic Press, Inc., New York.
31. **Lillehoj, H. S.** 1987. Effects of immunosuppression on avian coccidiosis: cyclosporin A but not hormonal bursectomy abrogates host protective immunity. Infect. Immun. **55**:1616–1621.
32. **McNamara, M. K., R. E. Ward, and H. Kohler.** 1984. Monoclonal idiotope vaccine against *Streptococcus pneumonia* infection. Science **228**:1325–1326.
33. **Miller, G. A., B. S. Bhogal, A. C. Anderson, E. J. Jessee, R. McCandliss, M. Likel, J. Strasser, S. Strausberg, and R. Strausberg.** 1989. Application of a novel recombinant *Eimeria tenella* antigen in a vaccine to protect broiler chickens from coccidiosis. Prog. Clin. Biol. Res. **37**:117–130.
34. **Mossmann, T. R., H. Cherwinski, M. W. Bond, M. A. Gredlin, and R. L. Cottmann.** 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. **136**:2348–2357.
35. **Murray, H. W., H. Masur, and J. S. Keithly.** 1982. Cell mediated immune responses in experimental visceral leishmaniasis. I. Correlation between resistance to *L. donovani* and lymphokine generating capacity. J. Immunol. **129**:344–350.
36. **Murray, H. W., B. Y. Rubin, and C. D. Rothermel.** 1983. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes: evidence that interferon-gamma is the activating lymphokine. J. Clin. Invest. **72**:1506–1510.
37. **Nathan, C. F., T. J. Pendergast, M. E. Weibe, E. R. Stanley, E. Platzer, H. G. Remold, K. Welte, Y. B. Rubin, and H. W. Murray.** 1984. Activation of human macrophages: comparison of other cytokines and interferon-gamma. J. Exp. Med. **160**: 600–605.
38. **Palladino, M. A., S. P. Lerman, and G. J. Thorbecke.** 1978. Requirements for induction of delayed type hypersensitivity in the chicken. Dev. Comp. Immunol. **2**:121–129.
39. **Rose, M. E.** 1978. Immune responses of chickens to coccidia and coccidiosis, p. 297–336. *In* P. L. Long, K. N. Boorman, and B. M. Freeman (ed.), Avian cocidiosis. British Poultry Science, Ltd., Edinburgh.
40. **Rose, M. E., H. S. Joysey, P. Hesketh, R. K. Grencis, and D. Wakelin.** 1988. Mediation of immunity to *Eimeria vermiformis* in mice by L3T4$^+$ T cells. Infect. Immun. **56**:1760–1765.
41. **Sacks, D. L., K. M. Esser, and A. Sher.** 1982. Immunization of mice against African trypanosomiasis using anti-idiotypic antibodies. J. Exp. Med. **155**:1108–1119.
42. **Sadick, M. D., F. P. Heinzel, V. M. Skigekane, W. L. Fisher, and R. M. Locksley.** 1987. Cellular and humoral immunity to *Leishmania major* in genetically susceptible mice after *in vivo* depletion of L3T4 T cells. J. Immunol. **139**:1303–1309.
43. **Schmatz, D. M., M. S. Crane, and P. K. Murray.** 1984. Purification of Eimeria sporozoites by DE-52 anion exchange chromatography. J. Protozool. **31**:181–183.
44. **Sharpe, A. H., G. N. Gaulton, K. K. McDade, B. N. Fields, and M. I. Greene.** 1984. Syngeneic monoclonal anti-idiotype can induce cellular immunity to reovirus. J. Exp. Med. **160**:1195–1206.
45. **Stein, K. E., and T. Soderstrom.** 1984. Neonatal administration of idiotype or anti-idiotype primes for protection against *Escherichia coli* K13 infection in mice. J. Exp. Med. **160**:1001–1011.
46. **Steinbeck, M. J., J. A. Roth, and M. L. Kaeberle.** 1986. Activation of bovine neutrophils by recombinant interferon-gamma. Cell. Immunol. **98**:137–144.
47. **Stern, J. J., M. J. Oca, B. Y. Rubin, J. L. Anderson, and H. W. Murray.** 1988. Role of L3T4 and Lyt2 cells in experimental visceral leishmaniasis. J. Immunol. **140**:3971–3977.
48. **Suzuki, Y., and J. S. Remington.** 1988. Dual regulation of resistance against *Toxoplasma gondii* infection by Lyt2$^+$ and Ly1$^+$, L3T4$^+$ T cells in mice. J. Immunol. **140**:3943–3946.
49. **Sy, M. D., B. A. Bach, Y. Dohi, A. Nisonoff, B. Benacerraf, and M. I. Greene.** 1979. Antigen and receptor driven regulatory mechanisms. I. Induction of suppressor T cells by anti-idiotypic antibodies. J. Exp. Med. **150**:1216–1224.
50. **Sy, M. S., A. R. Brown, B. Benacerraf, and M. I. Greene.** 1980. Antigen and receptor driven regulatory mechanisms. III. Induction of delayed type hypersensitivity to azobenzenearsonate by anti cross reactive idiotypic antibodies. J. Exp. Med. **151**: 896–906.
51. **Tarleton, R., and D. W. Scott.** 1987. Initial induction of immunity followed by suppression of responses to parasite antigens during *Trypanosoma cruzi* infection of mice. Parasite Immunol. **8**:579–589.
52. **Thomas, W. R., G. Morahan, J. D. Walker, and J. F. A. M. Miller.** 1981. Induction of delayed type hypersensitivity to azobenzenearsonate by a monoclonal anti-idiotype antibody. J. Exp. Med. **153**:743–747.
53. **Titus, R. G., R. Ceredig, J. C. Cerottini, and J. A. Louis.** 1985. Therapeutic effect of anti-L3T4 monoclonal antibody GK1.5 on cutaneous leishmaniasis in genetically susceptible BALB/c mice. J. Immunol. **135**:2108–2114.
54. **Urbain, J., M. Wikler, J. D. Franssen, and C. Collignon.** 1977. Idiotypic regulation of the immune system by the induction of antibodies against anti-idiotypic antibodies. Proc. Natl. Acad. Sci. USA **74**:5126–5130.