T Lymphocyte Reactivity to Glutamic Acid-Alanine-Tyrosine *In Vitro* Does Not Reflect Antibody Response *In Vivo*[1]

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ABSTRACT Mechanisms responsible for the differences in humoral immune response to GAT (a random linear amino acid polymer) were investigated in a line of chickens consisting of four sublines homozygous for *Ea-B* (B^1 or B^{19}) and high or low antibody response to GAT (Ir-GAT^H or Ir-GAT^L). Previous research provided evidence of chromosomal recombination between the serologically determined regions of the MHC (encoded by *B-F* and *B-G* genes) and the gene or genes that control immune response to GAT, but immune response to GAT did not seem to be mediated through differences in *B-L* gene products. In the present study, proliferation of GAT-primed T lymphocytes indicated that reactivity *in vitro* was not associated with antibody levels produced in the animal. Cell surface markers were identified by flow cytometry. Lymphocytes from *Ea-*B^{19} chickens that were Ir-GAT^L had a higher percentage of suppressor T (CD8)-positive cells than did lymphocytes from Ir-GAT^H chickens. The *Ea-*B^1 chickens that were Ir-GAT^L had a higher percentage of CD4-positive lymphocytes than did chickens that were Ir-GAT^H. This may indicate that low response to GAT in the *Ea-*B^{19} chickens, but not in *Ea-*B^1 chickens, is mediated by CD8-positive cells. The ability of antigen-presenting cells (APC) to process and present GAT to antigen-primed T lymphocytes was tested *in vitro*. Measurements of lymphocyte proliferation indicated that, within the *Ea-*B^1 blood type, APC from Ir-GAT^L chickens produced higher ($P < .05$) stimulation of both GAT low- and GAT high-responder lymphocytes. It is possible that, between the two *B* blood types, there are different mechanisms responsible for the differential response to GAT.

(*Key words*: immune response, glutamic acid-alanine-tyrosine, genetic differences, chickens, major histocompatibility complex)

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INTRODUCTION

Although Ir-GAT is linked to the B blood group in chickens, Pevzner *et al.* (1978) reported the recombination between the gene or genes responsible for immune response to GAT (Ir-GAT^H or Ir-GAT^L, detected as high or low antibody production) and genes coding for the serologically detectable MHC antigens (*Ea-B*). The ability, or lack thereof, in mice to produce antibody after challenge with particular amino acid polymers has been mapped to the immune response region (*I*) of the MHC (McDevitt *et al.*, 1972). Based on structural and functional homology, the gene products of the *B-L* region of the chicken MHC are the equivalent of the mouse immune response region genes (Hála *et al.*, 1981). This suggested that the recombination between Ir-GAT and the

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serologically detectable MHC gene products may have reflected recombination within the chicken MHC.

Attempts to detect chromosomal recombination within the MHC of the line with recombination between Ir-GAT and *Ea-B*, as expressed by a difference in cell surface protein (*B-L*) have not supported this hypothesis. Extensive reciprocal immunizations between Ir-GAT^H and Ir-GAT^L chickens with *B-L* antigen-positive cells have not resulted in antibody that could discriminate between GAT phenotypes (Steadham, 1991). Restriction fragment length polymorphisms generated by probing S1 line DNA with a Class II (*B-L* α) gene probe (Pitcovski *et al.*, 1989) or a Class I (*B-F*) gene probe (Chen and Lamont, 1992) have been associated with *Ea-B* differences, but not with Ir-GAT differences. Results indicate that, although the humoral response to GAT may be linked to the chicken MHC (Benedict *et al.*, 1975), the response may not be controlled by traditional Class I or Class II genes within the MHC.

The importance of Ir-GAT as a marker for disease resistance has been well investigated in the chicken. For example, incidence of Marek's disease in Marek's disease virus (MDV)-challenged chickens is low in the S1 line chickens that have the B^1B^1 blood type compared with the $B^{19}B^{19}$ blood type. Within the B^1B^1 blood type, chickens that are high responders to GAT are yet more resistant to MDV challenge (Pevzner *et al.*, 1981; Steadham *et al.*, 1987). This effect is also seen when more susceptible chickens [such as the S1 line ($B^{19}B^{19}$) chickens] are challenged with MDV; that is, the Ir-GAT^H chickens within the blood type are more resistant to incidence of the disease than Ir-GAT^L chickens. Pevzner *et al.* (1989a, b) conducted a divergent selection experiment within two types of commercial chickens (broilers and layers). After development of sublines that were low or high antibody responders to GAT challenge, the chickens were tested for their resistance to poultry pathogens (MDV, Rous sarcoma virus, and *Staphylococcus aureus*). The lines selected for high antibody response to GAT were more resistant to incidence of disease or the effects of the disease than were the low-responder lines. Equally important, selection for high GAT response did not result in any significant change in performance characteristics.

Although the results of selecting for high GAT response could result in healthier chickens, the underlying immunological mechanisms are poorly defined. The purpose of the present study was to examine some of the cellular reactions that contribute to immune response.

MATERIALS AND METHODS

Genetic Stocks

The S1 line of chickens originated from a cross of two commercial inbred lines (Nordskog *et al.*, 1973). Since 1978 the line has been maintained in sublines selected for homozygous *Ea-B* serotype (B^1B^1 or $B^{19}B^{19}$) and for humoral immune response (high or low) to the amino acid polymer GAT (Pevzner *et al.*, 1978). The inbreeding coefficients of the S1 sublines were approximately .52 (Nordskog and Cheng, 1988). Chickens used were of both sexes and between 12 and 16 wk of age unless otherwise noted.

Lymphocyte Culture

In vitro proliferation of T lymphocytes induced by GAT was assayed by a procedure modified from Vainio *et al.* (1988). Blood was collected 14 days after chickens were primed by intramuscular GAT immunization (250 μg GAT in .5 mL of .01 *M* PBS pH 7.2 emulsified in .5 mL of complete Freund's adjuvant). All immunizations were done with the same lot number of GAT[3] and complete Freund's adjuvant.[4] Peripheral blood lymphocytes (PBL) were separated from whole blood by centrifugation over Histopaque 1077[5] density gradient. The lymphocyte fraction was aseptically collected and washed three times in culture medium (RPMI 1640 supplemented with 2 m*M* L-glutamine, 100 U/mL penicil-

[3]Miles Laboratories, Elkhart, IN 46515.
[4]Gibco Laboratories, Grand Island, NY 14072.
[5]Sigma Chemical Co., St. Louis, MO 63178-9916.

lin, 100 μg/mL streptomycin, 5% heat-inactivated normal chicken serum, and 5 × 10^{-5} *M* 2-mercaptoethanol). After the last wash, the cells were resuspended in medium and the concentrations were adjusted to 1 × 10^7 cells per milliliter.

Cell suspensions (100 μL) were added to sterile, flat-bottomed, 96-well tissue culture plates.[6] Each well received an additional 100 μL of medium with or without GAT such that the final GAT concentration was 0, 1, or 10 μg per well. After a 3-day incubation at 41 C in a humidified, 5% CO_2 incubator, proliferation was measured by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

MTT Proliferation Assay

The T cell proliferation *in vitro* was determined by a colorimetric assay based on the cleavage of MTT by living cells (Mosmann, 1983). After a 3-day incubation, 20 μL of MTT stock solution (10 mg/mL MTT in .01 *M* PBS, pH 7.2) was added to each well of the cultured cells. The plate was returned to the incubator for additional incubation. At the end of 3 h, 150 μL of medium was removed, without disturbing the cells, from each well and 150 μL of acid-isopropanol (3.3 mL of 12 *N* HCl/L isopropanol). The plate was then vortexed to ensure lysis of the cells and to dissolve the cleaved MTT. Absorbance at 570 nm was determined for each well on an ELISA plate reader.[7] Data presented represent the mean absorbance at 570 nm of quadruplicate wells cultured under the same conditions.

Fluorescent Labeling of Cultured Cells

Cell-surface phenotypes of cultured cells receiving secondary exposure to GAT *in vitro* were determined by flow cytometric analysis of fluorescent antibody-labeled cells. Cells collected for analysis were cultured as described. Nonadherent cultured cells were placed in siliconized glass tubes and washed once in cold (4 C) wash buffer (1.1 × Dulbecco's PBS plus 2% of heat-inactivated fetal calf serum and .1% NaN_3) before labeling. The harvested cultured cells were resuspended in 100 μL of primary antibody dilution (anti-CD4 or anti-CD8) or wash buffer only (for controls). The cells were incubated for 30 min at 4 C. After incubation, the cells were washed three times with cold (4 C) wash buffer, and the second antibody was added.

The primary antibodies were monoclonal antibodies (as hybridoma culture supernatants) obtained from two sources. The anti-CD8 was a gift from Hyun Lillehoj (USDA, Agricultural Research Service, Beltsville, MD 20705) and was used at a dilution (in wash buffer) of 1:10. The anti-CD8 antibody identifies a population chicken T lymphocytes of the cytotoxic or suppressor functional type (Lillehoj *et al.*, 1988). The anti-CD4 monoclonal antibody (also used at 1:10 dilution) was a gift from Chen-lo Chen (University of Alabama, Birmingham, AL 35294). The anti-CD4 antibody identifies a molecule that is the avian homolog of the surface marker on T cells of the helper phenotype (Chan *et al.*, 1988). The secondary antibody was fluoroisothiocyanate-conjugated rabbit anti-mouse IgG $F(ab')_2$ fragments diluted 1:100 with wash buffer.

Washed cells were resuspended in 100 μL of the second antibody dilution and incubated for an additional 30 min at 4 C. Controls received no primary or secondary antibody or received secondary antibody only. After the second incubation, the cells were washed three times with cold (4 C) wash buffer. After the last wash, the cells were fixed by first suspending the cells in 200 μL of 1.1 × Dulbecco's PBS with .1% NaN_3 and then adding 200 μL of paraformaldehyde solutions (2% paraformaldehyde in 1.1 × Dulbecco's PBS with .1% NaN_3). Samples were refrigerated in the dark until analyzed by using flow cytometry.

Labeled cells were analyzed on an EPICS Profile I flow cytometer.[8] Lymphocyte populations were selected for fluorescence analysis on the basis of forward light scatter versus side light scatter (size versus granularity) histograms. The analyzed lym-

[6]Flow Laboratories Inc., McLean, VA 22102.
[7]Model ELISA Reader, Fisher Scientific Co., Itasca, IL 60143.
[8]Coulter Co., Hialeah, FL 33012.

phocyte populations represented an average of 84% of the cultured cells analyzed through the flow cytometer. Results were recorded as percentage fluorescence-positive cells (within gated fluorescence window) of 4×10^3 cells tested.

In Vitro *Antigen-Presenting Cell Assay*

Plastic-adherent cells from Histopaque 1077-isolated PBL were used as antigen-presenting cells (APC) for *in vitro* stimulation of *in vivo*-primed T cells. Adherent monolayer cells are primarily monocyte-macrophage cells (Chu and Dietert, 1989) and were prepared by a method used by Vainio *et al.* (1988). Peripheral blood lymphocytes were first isolated from whole blood of GAT-primed chickens by gradient centrifugation over Histopaque 1077. The mononuclear cell layer was collected, washed three times in RPMI 1640 (supplemented with 2 m*M* L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 5% heat-inactivated normal chicken serum, and 5×10^{-5} *M* 2-mercaptoethanol), and the cell concentration was adjusted to 5×10^6 cells per milliliter. The cell dilutions (200 μL per well) were placed into 96-well plates and incubated for 3 h in a humidified, 41 C, 5% CO_2 incubator to allow cell adherence to occur. Then the nonadherent cells were washed away (3×) with prewarmed (41 C) medium. Medium with GAT (0 to 10 μg per well) was added to the remaining adherent cells. The plates were returned to the incubator for a 5-h antigen pulse.

Following the antigen pulse, the APC cultures were prepared by thrice washing the adherent cells with supplemented medium (without GAT) to remove the noninternalized GAT. The responder lymphocytes from GAT-primed chickens were prepared as described previously and 1×10^6 cells in 200 μL of medium without antigen were added to each well. Responder cells and APC were combined within *Ea-B* type (to avoid mixed lymphocyte proliferative reaction) and were matched or mismatched by Ir-GAT type. The cultures were incubated for 3 days before proliferation was determined by the MTT proliferation assay. Results were converted to a stimulation index by dividing the mean absorbance of wells with GAT by the mean absorbance of the same cultures without GAT. The resulting value was multiplied by 100 and subtracted from 100. Thus, index scores above zero indicated proliferation above control values, and negative values indicated suppression below control values. The chickens used for these assays were hens over 52 wk of age.

Statistical Analysis

Where significant differences are reported, analyses of variance were performed on assay results by using the traits under consideration (e.g., *Ea-B* or Ir-GAT), sex, and GAT concentrations as sources of variation. Differences between means were tested by Duncan's multiple range test or *t* test (SAS Institute, 1985).

RESULTS

Table 1 presents the results of *in vitro* proliferation (mean absorbance at 570 nm) for the four *Ea-B* and Ir-GAT combinations. There were no statistically signifi-

TABLE 1. Mean proliferation response (absorbance at 570 nm) of T lymphocytes, from birds of sublines characterized for *Ea-B* and Ir-GAT, receiving secondary GAT exposure *in vitro*

			GAT concentration (μg per well)		
Ea-B	Ir-GAT[1]	n	0	1	10
$B^{19}B^{19}$	High	16	.78 ± .06[a]	.81 ± .06[a]	.84 ± .06[a]
$B^{19}B^{19}$	Low	16	.64 ± .03[b]	.66 ± .03[b]	.68 ± .03[b]
B^1B^1	High	16	.51 ± .04[b]	.51 ± .04[c]	.57 ± .04[bc]
B^1B^1	Low	16	.54 ± .05[b]	.54 ± .04[bc]	.54 ± .04[c]

[a–c]Means ± SE within a column with no common superscripts differ significantly in absorbance ($P < .05$).

[1]Ir-GAT = phenotypic immune response to GAT.

TABLE 2. Analyses of variance of factors influencing *in vitro* proliferative response *in vivo*-primed T-cells within GAT concentration of medium

	GAT concentration (μg per well)					
	0		1		10	
Source of variation	df	MS	df	MS	df	MS
EaB type	1	.53***	1	.71***	1	.66***
Ir-GAT type	1	.05	1	.06	1	13*
Ea-B × Ir-GAT	1	.11	1	.14*	1	.06
Error	60	.03	60	.03	60	.03

*$P < .05$.

***$P < .001$.

cant differences between GAT concentrations within *Ea-B* or Ir-GAT type. Within a concentration of GAT, there were differences by *Ea-B* and Ir-GAT type. These differences reflect the different ability of cells of a particular haplotype to proliferate under these particular culture conditions. At both concentrations of GAT, and in cultures without GAT, cells from $B^{19}B^{19}$ chickens that were high responders to GAT (Ir-GAT^{H}) had the highest mean absorbance at 570 nm.

The *Ea-B* and Ir-GAT type of the chickens were used as sources of variation in an analysis of variance (Table 2). Sex of the donor was not a significant source of variation so sex was omitted from the model. At each concentration of GAT, the *Ea-B* type of the donor was a significant source of variation ($P < .001$) with proliferation of $B^{19}B^{19}$ cells being higher than $B^{1}B^{1}$. At the highest GAT concentration (10 μg per well), Ir-GAT was a significant source of variation ($P < .05$).

Table 3 presents the mean percentage of CD4-positive lymphocytes for the three GAT concentrations used to culture cells from the four sublines. The percentage of positive cells did not differ within subline when the concentration of GAT in the culture medium changed. There were significant differences ($P < .05$) between sublines associated with *Ea-B* type. Cultures from $B^{19}B^{19}$ chickens generally had a higher percentage of CD4-positive cells. If results were analyzed within *Ea-B* type, there was no significant difference in percentage CD4-positive cells between Ir-GAT^{H} or Ir-GAT^{L} samples from $B^{19}B^{19}$ chickens. Within the $B^{1}B^{1}$ blood type, the cultures from Ir-GAT^{L} chickens had a higher percentage of CD4-positive cells ($P < .05$) if the cells were cultured at 10 μg GAT per well. Sex of the donor chicken was never a significant source of variation in percentage CD4-positive cells in any instance.

Table 3 also presents the mean percentage of CD8-positive cells for the four S1 sublines. The percentage CD8-positive did not differ within subline by GAT concentration; there were differences between sublines at each concentration of GAT used in the culture medium. The cultures from $B^{19}B^{19}$ Ir-GAT^{L} chickens had the highest percentage of CD8-positive cells. At each concentration of GAT in the medium, *Ea-B* type was a significant

TABLE 3. Percentage of CD4- and CD8-positive (+) cells determined by flow cytometry after culture at one of three concentrations of GAT of *in vivo* primed lymphocytes from chickens of S1 sublines

		GAT concentration (μg per well)					
		0		1		10	
Ea-B	Ir-GAT[1]	CD4+	CD8+	CD4+	CD8+	CD4+	CD8+
$B^{19}B^{19}$	High	49.2 ± 5.3^{a}	17.7 ± 1.3^{b}	52.6 ± 3.9^{a}	17.1 ± 1.2^{b}	50.4 ± 4.0^{a}	16.5 ± 1.5^{b}
$B^{19}B^{19}$	Low	47.6 ± 4.1^{a}	22.1 ± 1.5^{a}	48.9 ± 3.9^{a}	22.1 ± 1.5^{a}	49.2 ± 3.6^{a}	22.8 ± 1.9^{a}
$B^{1}B^{1}$	High	31.5 ± 2.8^{b}	15.8 ± 1.0^{b}	36.0 ± 2.5^{b}	14.8 ± 1.2^{b}	32.4 ± 2.7^{b}	15.7 ± 1.0^{b}
$B^{1}B^{1}$	Low	40.5 ± 5.0^{ab}	15.4 ± 1.0^{b}	44.0 ± 4.9^{ab}	16.2 ± .9^{b}	44.0 ± 4.6^{a}	15.6 ± 4.6^{b}

^{a,b}Means ± SE within a column with no common superscripts differ significantly in percentage positive lymphocytes ($P < .05$).

[1]Ir-GAT = phenotypic immune response to GAT.

source of variation ($P < .05$). At the 1- and 10-μg GAT per well concentrations, Ir-GAT type of the donor chicken was a significant source of variation ($P < .05$). There was no difference in percentage CD8-positive cells on the basis of sex of the chicken. Analyzed within blood type ($B^{19}B^{19}$ or B^1B^1), there was a significant difference ($P < .05$) in percentage CD8-positive cells associated with Ir-GAT type of the donor chicken within the $B^{19}B^{19}$ blood type. Samples from $B^{19}B^{19}$ chickens that were Ir-GAT^L had a higher percentage of CD8-positive cells when cultured at either concentration of, or without, GAT. Within the B^1B^1 blood type, there were no differences associated with Ir-GAT type.

The APC from GAT-primed chickens were used to establish cultures that mixed or matched Ir-GAT types of APC and *in vivo* primed responder lymphocytes. Table 4 presents the mean stimulation index scores (percentage of control) for the eight culture combinations assayed. The ANOVA were calculated within *Ea-B* type. Within B^1B^1 blood type, the Ir-GAT type of the APC was a significant source of variation ($P < .05$). The APC from Ir-GAT^L chickens presented antigen better to responder lymphocytes of either Ir-GAT type than did the Ir-GAT^H APC. The Ir-GAT type of the responder lymphocytes was not a significant source of variation. Within the $B^{19}B^{19}$ blood type, the means were not significantly different.

DISCUSSION

Measurements of *in vitro* reactivity of antigen (GAT)-primed lymphocytes were undertaken to determine whether there were differences in proliferation in response to GAT that could account for the differences in humoral anti-GAT response in the whole animal. The Ir-GAT type of the responder cells was a significant source of variation in proliferation (as measured by MTT assay) only at the 10-μg per well concentration of GAT. Within a subline, there were no differences in proliferation between levels of GAT. The T cell reactivity to GAT is not likely to be the cause of the difference in antibody response in these chickens, in contrast with similar experiments conducted with mouse cells (Kimoto and Fathman, 1980; Gougeon and Theze, 1983). Gougeon and Theze (1983) used cells from GAT responder and nonresponder mouse lines as source of antigen-primed lymphocytes. Subsequent secondary stimulation *in vitro* resulted in proliferation of responder mouse cells above levels seen for cells in wells without GAT. Cells from nonresponder mice did not proliferate *in vitro*.

The proliferation assay results might have been different if exogenous interleukin 2 (IL-2) had been added to the medium. The IL-2 would have allowed for a longer incubation time for proliferation differences to occur, and there is a synergistic effect when antigen and IL-2 are used to induce proliferation. At present, however, there is no consistent public source of chicken IL-2 other than using conditioned medium (the medium into which mitogen-stimulated T cells have

TABLE 4. Mean (± SE) *in vitro* proliferation index score for different combinations of *in vivo*-primed antigen-presenting cells (APC) and responder lymphocytes[1]

APC type		Responder type		n	Mean score
B^1B^1	Ir-GAT^H	B^1B^1	Ir-GAT^H	27	−2.1 ± 1.2
		B^1B^1	Ir-GAT^L	9	−3.8 ± 2.9
B^1B^1	Ir-GAT^L	B^1B^1	Ir-GAT^H	9	3.6 ± 1.6
		B^1B^1	Ir-GAT^L	3	6.6 ± 2.3
$B^{19}B^{19}$	Ir-GAT^H	$B^{19}B^{19}$	Ir-GAT^H	14	−1.5 ± 1.1
		$B^{19}B^{19}$	Ir-GAT^L	9	−2.4 ± 1.1
$B^{19}B^{19}$	Ir-GAT^L	$B^{19}B^{19}$	Ir-GAT^H	14	.1 ± 2.4
		$B^{19}B^{19}$	Ir-GAT^L	9	1.7 ± 1.9

[1]Ir-GAT^H = high phenotypic immune response to GAT. Ir-GAT^L = low phenotypic immune response to GAT.

secreted IL-2), and mammalian IL-2 is not biologically active on chicken lymphocytes (Schauenstein *et al.*, 1982). Conditioned medium not only contains IL-2 but also any mitogen that cannot be removed or inactivated and all other soluble factors secreted by the mitogen-stimulated cells.

The total proliferative response to secondary GAT exposure *in vitro* may not be as significant an indicator of antibody-producing potential as the functional type of cells that proliferate in response to the antigen. For example, cell numbers would not have to increase substantially to change the response if all the cells proliferating were antigen-specific and of either the suppressor or helper functional type (bearing CD8 or CD4 surface molecules, respectively). Small changes in populations of CD4- and CD8-positive cells can result in major differences in immune reactivity. Hála *et al.* (1991) has reported differences in basal levels of CD4 and CD8 lymphocytes in congenic lines of chickens that differ in their resistance to Rous sarcoma virus-induced tumors and in their humoral immune response to various antigens.

Gougeon and Theze (1983) demonstrated *in vitro* T cell proliferation in response to GAT in nonresponder mice by first deleting the suppressor T cells from the cell cultures. Pierce *et al.* (1988) showed that T cell populations that were induced in GAT nonresponder mice were specific for the antigen and had the phenotypic marker (CD8) of suppressor cells. Nonresponse to GAT in mice is seemingly mediated through antigen-specific suppressor T cells. In both of the previous examples, T cells from nonresponder mice could respond to GAT under the appropriate conditions; thus, nonresponse was not due to inability of the cells to recognize antigen.

The percentage of CD4- and CD8-positive cells in the present study did not change as a result of different concentrations of GAT in the culture medium, but there were differences resulting from being cultured. Freshly collected lymphocytes from S1-line chickens have been assayed to determine the percentage of CD4 and CD8 populations (Munns, 1990). Cells from B^1B^1 Ir-GAT^H chickens had the highest percentage of CD4 expression, and B^1B^1 Ir-GAT^L chickens were the lowest for percentage CD8 expression, with no significant differences between the other S1 sublines for either marker. The cultured cells from B^1B^1 Ir-GAT^H chickens had the lowest percentage of CD4-positive cells, and cells from $B^{19}B^{19}$ Ir-GAT^L chickens had the highest number of CD8-positive cells.

It is possible that there are different mechanisms responsible for the differential humoral immune response to GAT within the two *Ea-B* types of the S1 line. The sublines within the S1 line originally arose from the same genetic group, but some selection had taken place already within some of the sublines. Some of the ancestors of the current B^1B^1 sublines were drawn from populations that had been previously selected for antibody response to *Salmonella pullorum* (Pevzner *et al.*, 1977, 1978). Within the $B^{19}B^{19}$ blood type, low response to GAT could be mediated by induction of suppressor T cells (CD8-positive). Within the B^1B^1 blood type, low response to GAT could be due to a completely unrelated mechanism.

There are examples in which differences in levels of antibody produced can be attributed to differences in the ability of APC to process and present antigen. Some lines of "Biozzi" mice produce low levels of antibody because of the inability of their APC to present antigen to responsive T cells (Adorini and Doria, 1981).

When lymphocytes from GAT-primed chickens were cultured with GAT-pulsed APC from Ir-GAT^H or Ir-GAT^L chickens, the APC from the Ir-GAT^L chickens were better able to stimulate the responder cells, regardless of their GAT response phenotype. If APC defects were responsible for the differences seen in humoral immune response to GAT, the expectation would be that the APC from high-responder chickens would be more capable of presenting antigen than APC from low-responder chickens. The APC assayed *in vitro* did not support the hypothesis that there was a defect in antigen presentation in the S1 low-responders. Defective antigen presentation is not always the cause of low antibody production. Of the five different lines of "Biozzi" mice

(selected for divergent antibody production to different antigens) tested, deficient antigen presentation was a likely cause of low antibody production in only two of the lines (Biozzi *et al.*, 1984; Mouton *et al.*, 1984).

Humoral immune response to GAT in chickens may be determined at levels of cell interaction other than those mediated through Class II MHC molecules. There are other cellular interactions that could result in differences in humoral antibody response. For example, there could be differences in antigen processing or presentation by antigen-presenting cells (Adorini and Doria, 1981) or differences in effector cell populations (Hála *et al.*, 1991). The response and phenotypic markers of antigen-primed lymphocytes secondarily exposed to GAT *in vitro* was assayed to clarify the nature of antibody response to GAT in chickens.

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