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Natural Killer Cell Activity in Chickens: Target Cell Analysis and Effect of Antithymocyte Serum on Effector Cells

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A battery of lymphoid and nonlymphoid cells was examined for susceptibility to lysis by natural killer cells of chickens. Several susceptible targets were recognized, and most susceptible among these were cells of line LSCC-RP9, derived from a lymphoid tumor induced by Rous-associated virus 2. The natural killer reactivity against LSCC-RP9 target cells did not appear to be directed against an antigen(s) induced by Rous-associated virus 2 because other lymphoid and nonlymphoid cells infected with this were resistant to lysis in vitro by natural killer cells. The effector cells of natural killer reactivity in chickens were refractory to treatment with potent anti-T-cell and anti-B-cell sera. Inoculation of Marek's disease virus in line 15 × 7 chickens resulted in enhanced natural killer cell activity, and the effector cells of this enhanced activity were also resistant to anti-T-cell serum.

Studies within the last decade have revealed that natural killer (NK) cells may be an important first line of defense against neoplasms. NK cells, present in unprimed individuals, can lyse a variety of target cells in vitro. Mammalian NK cells, most widely studied in mice, are bone marrow-derived, Fc receptor-bearing cells distinct from conventional T and B lymphocytes or macrophages, although there is some evidence that NK cells may be precursors of T cells (8). NK cells can be detected in various lymphoid cell reservoirs except the thymus. There is wide variation in the extent of NK cell expression between genetic strains of mice and rats, although the mechanisms that regulate NK activity are not as yet fully understood. Nude mice have high NK cell levels (10); thus, unlike conventional T-cell immunity, NK expression is not regulated by the thymus.

There is a wide variety of syngeneic, allogeneic, or xenogeneic lymphoid or nonlymphoid target cells that are susceptible to NK cell lysis in vitro. However, most studies have used lymphoid cells of tumor origin as target cells for in vitro assays of NK reactivity. NK cell cytotoxicity appears to be specifically directed against a wide range of cell surface antigens. Initial evidence suggested that relevant surface antigens on target cells may be associated with expression of endogenous or exogenous tumor virus (10, 12). However, later genetic studies revealed that expression of high levels of NK activity was a dominant phenotypic trait that partially segregated with the *H-2* locus and was not consistently related to tumor virus exposure (19). The specificity of NK cells derived from animals stimulated with viruses may be somewhat different from that of NK cells obtained from unstimulated animals (28).

We and others have recently recognized that chickens may also have NK cells (13, 27). We detected NK activity in chickens of several genetic lines by reacting spleen cells with MDCC-MSB1 target cells in a 4-h ^{51}Cr release test. Our initial work indicated that, as in rodents, NK cell expression in chickens was age dependent; detectable activity was minimal in chicks during the first few weeks after hatching but increased as the chickens became older (27). The effector cells of the avian activity were nonadherent and resided predominantly in the spleen. In this study, our objectives were to analyze additional target cells for NK cell susceptibility and to further characterize the effector cells in unstimulated and virus-infected chickens.

MATERIALS AND METHODS

Chickens. Chickens of lines P, 6_3, and 15 × 7 were from the flocks maintained at this laboratory. The parent stock was hatched and raised in an environment of biologically filtered air and was free of detectable exposure to various common poultry pathogens, including exogenous ribonucleic acid tumor viruses, Marek's disease virus (MDV), herpesvirus of turkeys, avian adenoviruses, *Salmonella pullorum*, *S. gallinarum*, *Mycoplasma gallisepticum*, *M. synoviae*, reovirus, Newcastle disease virus, infectious laryngotracheitis virus, infectious bronchitis virus, avian encephalomyelitis virus, and reticuloendotheliosis virus. The progeny chickens used were wing banded at hatching and immediately placed in Horsfall-Bauer-type cages under negative filtered-air pressure. The chickens were held in isolation until used. Because uninoculated control chickens were often housed in Horsfall-Bauer

cages adjacent to the ones containing chickens experimentally infected with MDV, particular care was taken to ensure freedom of the control chickens from inadvertent exposure to MDV. At the end of the experiment, all chickens were necropsied and examined for gross lesions, and sections of vagus nerve, brachial and sciatic plexuses, and gonads were fixed in formaldehyde solution and examined for microscopic lesions of Marek's disease (MD) (18). In addition, the chickens were bled periodically, and sera were examined by the agar gel precipitin test for antibody to MDV. In no instance was MD diagnosed in control chickens.

MD immune effector cells. Chickens of line 15 × 7 were inoculated at 5 to 11 days of age with a cloned tissue culture preparation of the JM strain of MDV (clone 111S; 24). Ten thousand plaque-forming units of MDV was injected intraabdominally into each chicken. Spleens were obtained 7 to 10 days after inoculation and used as a source of immune effector cells. Individual or pooled spleens were disrupted by mechanical pressure with the flat end of a plunger of a 10-ml syringe, and single cells released into the overlying medium (1.1 × solution of Dulbecco phosphate-buffered saline at pH 7.4, containing 2% fetal bovine serum [DPBS]) were clarified on a Ficoll-Hypaque gradient at a specific gravity of 1.09.

Target cells. The cell lines or transplants developed from the lymphoid leukosis-sarcoma group of viruses (Table 1) included: LSQC-16Q (17), LSCC-RP9 (W. Okazaki, R. L. Witter, C. Romero, K. Nazerian, J. M. Sharma, A. Fadly, and D. Ewert, Avian Pathol., in press), 1104-X5 (4, 5), 1104-B-1 (4, 5), LSCC-BK3 (6), P-1 (K. Nazerian, W. Okazaki, and H. G. Purchase, unpublished data), LSCT-RP6 (Okazaki et al., in press), LSCC-RP12 (Okazaki et al., in press), and hamster clones 2 and 3292 (20). The cells of LSCT-RP6 were maintained by serial inoculation in line 15 × 7 chickens (Okazaki et al., in press). The chickens inoculated with LSCT-RP6 cells at 7 days of age developed tumors at the site of inoculation (pectoral muscles) and greatly enlarged livers within 1 to 2 weeks. Fresh single-cell suspensions of tumorous pectoral muscle or liver were used as target cells. Primary or secondary SPAFAS chicken embryo fibroblast (CEF) monolayer cells infected with the following lymphoid leukosis-sarcoma viruses were also tested as target cells (Table 1): Rous-associated virus 1 (RAV-1), RAV-2, RAV-49, Bryan high-titered Rous sarcoma virus–RAV-1 [BH-RSV(RAV-1)], and Schmidt-Ruppin RSV-2 (SR-RSV-2). Primary CEF monolayers were infected with 10^4 to 10^5 infectious units of the above viruses. After 5 days at 37°C, monolayer cells were subcultured; after an additional 5-day incubation at 37°C, the cells were used as target cells in the ^{51}Cr release assay. The titer of virus in supernatant fluids from secondary cell cultures ranged from 10^5 to 10^7 infectious units per ml.

The target cell lines derived from MD lymphomas (Tables 1 and 2) included MDCC-RP4 (K. Nazerian, W. S. Payne, L. F. Lee, and R. L. Witter, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, S294, p. 288), MDCC-SK3 (3), MDCC-RP2 (Nazerian et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979), and MDCC-MSB1 (1). The cell line QT-6 was derived from a methylcholanthrene-induced fibrosarcoma of quails (16). The SCT-RP10 line was a spontaneous T-cell lymphoma that originated in specific-pathogen-free chickens of line 7_1 (2). Cells of this transplantable tumor were kept frozen at −196°C and were thawed just before use as target cells. Most cell lines were maintained in vitro by serial subculture in RPMI 1640 medium (Flow Laboratories, Rockville, Md.) fortified with antibiotics (100 U of penicillin and 100 μg of streptomycin per ml) and 5 to 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). For lines LSCC-RP9, LSCC-RP12, 1104-X5, 1104-B-1, LSCC-BK3, and P-1, the above medium was further fortified with 2 to 5% chicken serum (GIBCO). The cells were used within 12 to 24 h of subculture.

^{51}Cr release assay. The NK and MD immune activity of effector cells was assayed in a 4-h ^{51}Cr release test. This test was performed according to the general procedures outlined previously (27) with modifications. The target cells suspended in DPBS were labeled for 45 min at 37°C with 250 μCi of ^{51}Cr, and 5 × 10^3 or 1 × 10^4 labeled target cells were allowed to react with various concentrations of effector cells in a total volume of 0.2 ml in wells of Falcon Microtest II plates with flat bottoms. After 4 h of incubation at 37°C on a rocker platform, the entire contents of each of three replicate wells in each treatment group were suspended in 1.3-ml portions of DPBS. The suspensions were centrifuged at 700 × *g* for 5 min, and the entire supernatant in each tube was counted for radioactivity in a Beckman 300 gamma irradiation counter (Beckman Instruments, Inc., Irvine, Calif.). The counts of replicate tubes were averaged. The standard error of counts of replicate samples within groups was less than 5% and is not shown with the data. The baseline control consisted of target cells mixed with normal chicken thymus cells in ratios corresponding to those of target and effector cells. The baseline lysis was usually 7 to 25%. Extensive testing revealed that thymus cells were not cytotoxic for the target cells, and ^{51}Cr release levels in target cell-plus-thymus cell mixtures were comparable to those in target cell suspensions incubated alone. The percentage of cytotoxicity was calculated by the following formula:

$$\% \text{ cytotoxicity} = \frac{\text{cpm in target cells mixed with effector cells} - \text{cpm incorporated in target cells} - \left(\frac{\text{cpm in target cells mixed with normal thymus cells}}{\text{cpm in target cells mixed with normal thymus cells}} \times 100\right)}{}$$

Specific ^{51}Cr release values were analyzed by Student's *t*-test.

Mitogenic assay. The general technique for this assay has been described in detail (14). Single-cell suspensions of spleens from normal chickens were stimulated in vitro with phytohemagglutinin-P (PHA; Difco Laboratories, Detroit, Mich.) and pulsed with [^{125}I]iododeoxyuridine (Amersham Corp., Arlington Heights, Ill.). Cells were assayed for incorporation of

TABLE 1. *Target cells examined for susceptibility to NK cell lysis*

Designation	Origin and characteristics	Reference	Susceptibility to NK cell lysis[a]
MDCC-RP4	Lymphoblastoid T-cell line derived from an MD transplant, MDCC-RP3; grows as a single-cell suspension	—[b]	–
MDCC-SK3	Lymphoblastoid cell line derived from an MD transplant, MDCT-NYM1	3	–
MDCC-RP2	Lymphoblastoid cell line derived from an MD lymphoma	—[b]	–
LSQC-16Q	Cell line of quail embryo fibroblasts infected with RSV (–)	17	–
QT-6	Methylcholanthrene fibrosarcoma of quail	16	–
LSCC-RP9	Lymphoblastoid cell line derived from the lymphoid leukosis tumor transplant LSCT-RP6	19	+
1104-X5	Attaching or adhering lymphoblastoid cell line derived from a bursal tumor induced by a subgroup A lymphoid leukosis virus (LLV)	4, 5	–
1104-B-1	Lymphoblastoid cell line derived from a bursal tumor induced by subgroup A LLV	4, 5	+
LSCC-BK3	Lymphoblastoid cell line derived from a bursal tumor induced by subgroup A LLV	6	–
P-1	Cell line obtained from SR-RSV-2-induced pig fibrosarcoma	Nazerian, Okazaki, and Purchase (unpublished data)	–
CEF/RSV-1	CEF cultures transformed by BH-RSV(RAV-1) (defective)		–
CEF/SR-RSV	CEF cultures transformed by SR-RSV-2		–
Hamster 3292	Clone of a continuous line of hamster cells transformed by BH-RSV	22	–
Hamster clone 2	Clone of a continuous line of hamster cells transformed by SR-RSV	22	–
SCT-RP10	T-cell line from a spontaneous lymphoma in line 7_1 chickens	2	–
LSCT-RP6	Cell transplant derived from RAV-2-induced tumor in line 15 × 7 chickens	Okazaki et al., in press	–
LSCC-RP12	Lymphoblastoid cell line derived from a lymphoid leukosis transplant, LSCT-RP6	Okazaki et al., in press	+
CEF/RAV-1	CEF cultures infected with RAV-1		–
CEF/RAV-2	CEF cultures infected with RAV-49		–
1104-B-1/RAV2	1104-B-1 cells infected with RAV-2		–

[a] In separate trials, one or more ^{51}Cr-labeled test target cells were reacted with effector cells at target cell/effector cell ratios of 1:100, 1:200, and 1:400. The cytotoxic reactivity of effector cells used in each trial was confirmed by reacting the effector cells with known susceptible target cells such as MDCC-MSB1 or LSCC-RP9. Each effector-plus-target combination was reacted in triplicate wells of Microtest II plates. Triplicate counts per minute of effector-plus-target combinations (test values) were compared (by Student's t-test) with triplicate counts of target cell-plus-normal thymus cell mixtures (background values). If, with a given target cell, test values were significantly higher ($P \leq 0.05$) than the background values in three repeat trials, the target cell was considered susceptible to lysis by effector cells.

[b] K. Nazerian, W. S. Payne, L. F. Lee, and R. L. Witter, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, S294, p. 288).

radioactivity after they were harvested onto glass filter papers (Reeve-Angel, Clifton, N.J.) with a MASH II automatic sample harvester (Microbiological Associates, Bethesda, Md.). Filters were counted for radioactivity in a Beckman 300 gamma irradiation counter.

Serology. Antisera to chicken thymus cells (ATS) and bursal cells (ABS) were prepared in rabbits as described earlier (24). Rabbits were given three serial biweekly intravenous injections of single-cell suspensions of thymus or bursa of 3-week-old chickens. Sera collected 7 to 10 days after the last injection were repeatedly cross-absorbed with thymus or bursa cells to remove nonspecific activity. Absorbed ATS and ABS were highly reactive against respective homologous but not against heterologous cells in a complement-dependent antibody cytotoxicity test and an indirect surface immunofluorescent (IF) test (24, 25). An MD tumor-associated surface antigen (MATSA) an-

TABLE 2. *Comparison of LSCC-RP9 and other target cells susceptible to NK cell lysis*

Expt no.	Target cell	% Cytotoxicity of effector cell prepn no.[a]					
		1			2		
		1:100[b]	1:200	1:400	1:100	1:200	1:400
1	1104-B-1	0	2.7	5.3	0	2.2	2.9
	LSCC-RP9	2.4	7.0	11.0	2.6	4.7	8.7
2	LSCC-RP12	4.9	5.1	8.7	0	4.7	5.1
	LSCC-RP9	14.0	17.5	26.2	7.3	11.8	17.1
3	MDCC-MSB1	3.2	6.7	ND[c]	9.1	13.2	ND
	LSCC-RP9	14.7	18.4	ND	34.8	36.4	ND

[a] Effector cells were obtained from spleens of 10- to 14-week-old line P or 6_3 chickens.
[b] Target cell/effector cell ratio.
[c] ND, Not done.

tiserum was also prepared in rabbits by two serial intravenous injections with MDCC-MSB1 cells (30). Serum obtained 2 weeks after the second injection was exhaustively absorbed with erythrocytes and lymphocytes from normal chickens until reactivity against normal cells (but not against MD lymphoma cells) disappeared.

The antibody- and complement-dependent cytotoxicity test was done by labeling target cells with ^{51}Cr and incubating 10^5 labeled cells with appropriate dilutions of antiserum and guinea pig complement (Difco) as described in detail previously (24). The IF staining of viable cells was performed on ice according to the standard procedures. Cells were allowed to react for 25 min with the appropriate dilution of rabbit antiserum, washed, and then allowed to react for 25 min with goat anti-rabbit gamma globulin conjugated with fluorescein isothiocyanate (Mills Yeda Limited, Elkhart, Ind.). Stained preparations were washed and examined with a Leitz fluorescence microscope with vertical illuminating system and ×63 oil immersion objective.

In certain experiments, the effect of ATS or ABS treatment on effector cells was studied. Ten million effector cells were allowed to react at room temperature with a 1:5 final dilution of antiserum. After 45 min of incubation, the cells were pelleted and the supernatants were drained off. The pelleted cells were suspended in 0.2 ml of an appropriate dilution (usually 1:10) of guinea pig complement and incubated at room temperature for 45 min. At the end of the incubation period, the cells were washed three times with DPBS. In some experiments in which effector cells of natural cytotoxicity were treated with ATS and ABS, the specificity of cytotoxic activity of these antisera was monitored by simultaneously treating normal spleen cells with the antisera and stimulating treated and untreated cells with a T-cell mitogen (PHA). Pretreatment with ATS but not with ABS abrogated the mitogenic response. We showed previously that in an IF test, an average of 52.4% (range, 44.0 to 61.4%) and 22.4% (range, 14.8 to 26.5%) of normal spleen cells reacted with ATS and ABS, respectively (25).

RESULTS

Target cell analysis. Among several target cells examined previously for susceptibility to NK cell lysis, cells of line MDCC-MSB1 were found to be most susceptible (27). Even with this target, the levels of in vitro cytotoxicity were usually quite low. Therefore, in this study, efforts were continued to screen additional types of target cells. Effector cells obtained from the spleens of 10- to 14-week-old line 6_3 or P chickens were reacted with a battery of target cells of lymphoid and nonlymphoid origin. Results of target cell analysis (Table 1) indicated that cells of lines LSCC-RP9, 1104-B-1, and LSCC-RP12 were lysed by effector cells; other target cells examined were refractory. The relative susceptibilities of three susceptible targets were compared (Table 2). Cytotoxicity levels were consistently higher with LSCC-RP9 cells than with 1104-B-1 or LSCC-RP12 cells. Table 2 also shows the comparison of cells of lines LSCC-RP9 and MDCC-MSB1. As noted above, MDCC-MSB1 target cells were used initially to detect NK cell activity in chickens (27). It was clear from the results in Table 2 that among those examined, LSCC-RP9 was the target cell most susceptible to lysis by NK cells. Two additional target cells found previously to be susceptible to NK cells, MDCC-HP1 and REV, were also assumed to be less susceptible than LSCC-RP9 cells, because levels of cytotoxicity against both of these targets were lower than those against MDCC-MSB1 cells (27).

The NK cell reactivity against LSCC-RP9 target cells did not appear to be directed against antigens induced by RAV-2, because other RAV-2-infected lymphoid cells (LSCT-RP6) and CEF cells were resistant to NK lysis in vitro (Table 1).

Effect of ATS and ABS on effector cells. Previously we had shown that the NK cell activity in chickens was not associated with adherent cells (27). Several trials were done to determine the effect of ATS treatment on NK cells. Two positive controls to monitor the efficacy of the stock ATS used were included: ATS was allowed

to react with (i) immune effector cells obtained from chickens 1 week after inoculation with MDV and (ii) normal spleen cells before they were examined for mitogenic response to PHA. Previous studies have shown that effector cells of MD immune cytotoxicity and of PHA-induced blastogenesis are T cells that can be readily inactivated by ATS (14, 24).

Pretreatment with ATS substantially reduced the cytotoxicity of immune effector cells and the blastogenic response of normal spleen cells, whereas there was little effect on cytotoxicity of spleen cells against LSCC-RP9 target cells (Table 3). In experiment 5 (Table 3), ATS plus complement substantially reduced the natural cytotoxicity of spleen cells. This result might suggest variability in response of certain NK cell preparations to ATS treatment, although in experiment 5, complement alone was also quite toxic for effector cells. In two of five experiments shown in Table 3, effector cells of NK cytotoxicity, immune cytotoxicity, and blastogenic activity were also pretreated with ABS and were found to be resistant. Thus, effector cells of immune and NK cytotoxicity could be differentiated: immune effector cells were inactivated by pretreatment with ATS, whereas NK cells resisted this treatment.

The immune effector cells used above were obtained by inoculating line 15 × 7 chickens with MDV at 6 days of age and collecting spleens 7 to 10 days later. Previous studies have shown that such treatment results in a T-cell-mediated immune response that peaks 1 to 2 weeks after MDV inoculation (26). We noted that at the peak of immune activity in virus-infected chickens, there was also some augmentation of cytotoxicity of spleen cells against LSCC-RP9 cells. Table 4 shows results of three experiments in which spleen cells from chickens exposed to MDV 1 week previously and from unexposed hatchmates were tested simultaneously against MDCC-MSB1 and LSCC-RP9 target cells. The cytotoxicity against both targets was substantially higher in virus-infected chickens than in uninoculated hatchmates. As noted previously (27), spleen cells of 2-week-old virus-free chickens had low cytotoxic reactivity agaisnt MDCC-MSB1 target cells (Table 4). Efforts were made to determine the nature of anti-LSCC-RP9 cytotoxicity enhanced by inoculation with MDV.

The antigenic specificity of immune cytotoxic reactivity against MDCC-MSB1 cells has not been defined. Circumstantial evidence suggesting association of this reactivity with MATSA was not supported by recent data (21). Because certain MD lymphoma cells and MDCC-MSB1 cells share MATSA, it was of interest to determine whether LSCC-RP9 cells also express this antigen. Specific anti-MATSA rabbit serum was

TABLE 3. *Effect of ATS and ABS on effector cells of NK cytotoxicity, immune cytotoxicity, and mitogenic response to PHA*

Expt no.	Target cell	Treatment of effector cells	% Cytotoxicity[a]		Mitogenic response of normal spleen cells[b]	
			Immune effectors[c]	NK cells[b]	PHA	No PHA
1	MDCC-MSB1	None	16.7	24.5	8,580	458
		ATS + complement	5.0	24.8	1,450	333
		ABS + complement	14.5	22.3	8,175	448
		ATS	15.6	20.1	8,787	323
		ABS	Not done	22.0	10,420	302
		Complement	16.4	23.7	9,033	420
2	MDCC-MSB1	None	17.6	4.5	3,940	164
		ATS + complement	7.6	4.6	491	133
		ABS + complement	17.7	6.0	4,437	187
		Complement	15.1	4.0	5,460	267
3	LSCC-RP9	None		7.4	3,731	417
		ATS + complement		13.5	1,623	314
		Complement		14.7	5,810	680
4	LSCC-RP9	None		32.4		
		ATS + complement		38.9		
		Complement		40.0		
5	LSCC-RP9	None		26.0	7,251	291
		ATS + complement		10.9	102	95
		Complement		15.5	6,169	365

[a] Target cell/effector cell ratio of 1:200.

[b] NK cells and cells for mitogenic response were from normal isolator-reared 10- to 14-week-old line P or line 6_3 chickens.

[c] Immune effector cells were from spleens of 2-week-old 15 × 7 chickens inoculated at 1 week with MDV.

TABLE 4. *Effect of MDV inoculation on cytotoxicity of spleen cells against MDCC-MSB1 and LSCC-RP9 target cells*

Expt no.	Effector cell donors[a]	% Cytotoxicity of effector cells against:					
		MDCC-MSB1			LSCC-RP9		
		1:50[b]	1:100	1:200	1:50	1:100	1:200
1	1 wk after MDV	2.0	4.6	10.2	13.7	15.3	26.7
	Uninoculated hatchmates	0	0	0	5.4	5.2	8.7
2	1 wk after MDV	5.5	3.3	6.3	5.2	8.3	11.5
	Uninoculated hatchmates	2.4	2.6	1.8	1.5	2.9	2.5
3	1 wk after MDV	2.2	4.7	8.2	11.0	16.6	24.7
	Uninoculated hatchmates	0.9	1.4	1.5	5.9	9.3	16.2

[a] Spleens of two to five chickens of line 15 × 7 were pooled. MDV was inoculated intraabdominally when animals were 6 days old.

[b] Target cell/effector cell ratio.

allowed to react against MDCC-MSB1, LSCC-RP9, and normal spleen cells in a complement-dependent antibody cytotoxicity test with ^{51}Cr-labeled target cells. The cells of LSCC-RP9 and normal spleen were refractory to treatment with anti-MATSA serum dilutions of 1:20 and 1:40, whereas treatment of MDCC-MSB1 cells with these two antiserum dilutions resulted in specific ^{51}Cr release of 57 and 45%, respectively. Additionally, in an IF test in which cells were first treated with a 1:10 dilution of rabbit anti-MATSA serum and then with fluorescein isothiocyanate-conjugated anti-rabbit goat gamma globulin, LSCC-RP9 cells failed to stain detectably, whereas simultaneously treated MDCC-MSB1 cells stained brilliantly. The above observations indicated that LSCC-RP9 cells did not express detectable MATSA and that the cytotoxicity of spleen cells from MDV-infected chickens against this target could not be explained on the basis of anti-MATSA immunity.

The nature of effector cells of anti-LSCC-RP9 activity was next examined. Chickens of line 15 × 7 were inoculated with MDV at 5 or 11 days of age, and spleen cells obtained 9 or 10 days after inoculation were pretreated with ATS and allowed to react against LSCC-RP9 target cells in a 4-h ^{51}Cr release assay. MDV inoculation enhanced cytotoxicity of spleen cells against LSCC-RP9 target cells, and the effector cells obtained from virus-infected or control chickens were refractory to ATS (Table 5). This result provided evidence that MDV inoculation generated two types of cytotoxic activity in chickens: first, a T-cell-mediated activity detectable with MDCC-MSB1 target cells, and second, a non-T-cell, presumably NK cell, activity most pronounced against LSCC-RP9 target cells.

DISCUSSION

The present study revealed that lymphoblastoid cells of an in vitro-propagating line, LSCC-RP9, derived from a tumor induced by RAV-2 were susceptible to avian NK cell lysis. The susceptibility of LSCC-RP9 cells to NK cell lysis exceeded that of all other susceptible targets recognized thus far. The LSCC-RP9 cells should serve as suitable targets for further studies characterizing NK activity in chickens.

In this and a previous study (27), we examined several categories of target cells for susceptibility to NK cell lysis. The target cells included lymphoid and nonlymphoid cells derived from spontaneous tumors, chemically induced tumors, and tumors originating in animals infected with herpesvirus or lymphoid leukosis viruses. No distinct pattern of NK cell susceptibility suggestive of antigenic specificity has emerged among these target cells. Because virus-associated antigens have been implicated in NK cell reactivity in rodents (10, 12, 22), attempts were made to determine whether the susceptibility of LSCC-RP9 cells was associated with antigens determined by infection with RAV-2. The LSCC-RP9 line was derived from a bursal lymphoma induced by infection of donor chickens with RAV-2, and the cells of this line continually produce RAV-2 (Okazaki et al., in press). No apparent association between RAV-2 and susceptibility to NK cell lysis could be established, because CEF cells infected with RAV-2 and LSCT-RP6 cells, from which LSCC-RP9 was derived, were resistant to lysis by NK effector cells. Indeed, another subline of LSCC-RP9, contaminated with RAV-1 and continually secreting RAV-2 and RAV-1, was also found to be resistant to NK cell lysis (27). Thus, the antigens determining the susceptibility of LSCC-RP9 to NK cell lysis did not seem associated with RAV-2, and the nature of these antigens was not clarified.

We have previously indicated that avian endogenous and exogenous leukosis viruses may not be directly involved in NK cell expression in chickens, because this activity in various genetic

TABLE 5. *Effect of ATS on effector cells induced by MDV in line 15 × 7 chickens*

Expt no.	Age (days) of effector cell donor		Treatment of effector cells	% Cytotoxicity vs LSCC-RP9	
	At MDV inoculation	When tested		1:100[a]	1:200
1		15	None	5.8	5.9
			ATS + complement	5.1	6.6
			ATS	2.8	4.4
			NRS[b] + complement	2.3	6.1
			Complement	5.3	5.6
	5	15	None	6.3	9.4
			ATS + complement	7.9	8.2
			ATS	6.6	5.5
			NRS + complement	6.3	8.6
			Complement	5.8	8.7
2		20	None	7.1	11.0
			ATS + complement	7.8	9.4
			Complement	6.5	12.3
	11	20	None	12.7	20.7
			ATS + complement	10.3	15.5
			Complement	16.7	19.5

[a] Effector cell/target cell ratio.
[b] NRS, Normal rabbit serum.

lines of chickens did not appear to correspond with the exposure of chickens to lymphoid leukosis viruses, and the susceptible target cells of line MDCC-MSB1 were free from any evidence of infection with endogenous or exogenous leukosis virus (27).

The characteristics of effector cells of mammalian NK activity have been widely studied, and there is evidence that morphologically NK cells fall into the category of small lymphocytes (11, 23). Although there is some controversy over the nature of cell surface markers expressed by NK cells (7), the recognition of high levels of NK activities in nude mice (10) has served to separate this cytotoxicity from the conventional T-cell-mediated immune response that develops after antigenic challenge. Mammalian NK cells are largely resistant to anti-T-cell serum, although antiserum partially inactivates NK cells from nude mice and from mice in which NK cell activity has been experimentally boosted (9, 28). Recently, the majority of NK cells from nude BALB/c mice and a certain proportion of NK cells from normal CBA mice reacted with monoclonal immunoglobulin M anti-Thy1-2 antibody (15). The partial expression of certain T-cell surface markers on mouse NK cells and the low but detectable affinity of human NK cells for sheep erythrocytes (29) suggested that NK cells may be of T-cell lineage. Our data indicated that avian NK cells resisted treatment with rabbit antiserum prepared against chicken thymocytes. This antiserum was highly reactive against mature T cells, because it abrogated the cytotoxic activity of MD immune effector cells and the blastogenic response of normal spleen cells to PHA. Our results revealed the non-T-cell nature of avian NK cells, and in this respect established a common characteristic or mammalian and avian NK cells. Lam and Linna (13) also noted that spleen cells from normal adult chickens, which protected young chickens against lethal effects of a transplantable tumor, were resistant to ATS.

It was of interest that MDV inoculation of 15 × 7 chickens enhanced the cytotoxicity of spleen cells against LSCC-RP9 targets. We have noted similar enhancement of NK reactivity by MDV in other genetic lines of chickens (J. M. Sharma, unpublished data). The NK activity in rodents can be boosted by injections with a variety of inocula including viruses, tumor cells, and reticuloendothelial system stimulants (9, 28, 31). The enhancement of NK activity in chickens by MDV is of particular interest because this virus is widespread in the avian population and causes a milignant lymphoproliferative disease. Further, MD is the only naturally occurring neoplasm of animals or humans that can be successfully prevented by vaccination. The possible involvement of NK cells in the disease response of susceptible or immunized chickens to MDV inoculation is currently under study in our laboratory.

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