

Pathogenesis and Characterization of Subgroup J Avian Leukosis Virus-Induced Histiocytic Sarcomas in Meat-type Chickens.

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Abstract

Histiocytic sarcomas (HS) are variably described in mammals and avians. The etiology and pathogenesis of these lesions is not clear in most cases. However, in chickens, a low incidence (1.1 – 5 %) of HS is consistently observed in subgroup J avian leukosis virus (ALV J) infections. The effect of ALV J viral factors (strain and dose) and host factors (genetic strain and age at infection) on the incidence of HS was evaluated in 2 experiments involving 449 meat-type chickens and 100 ADOL-Line-0 chickens (white leghorn strain free of endogenous retroviral *env* loci). Results indicated that only persistently viremic meat-type chickens inoculated at hatch develop HS with no effect of viral strain and/or dose. HS in chickens are primarily of splenic origin with frequent hepatic and renal metastases. Microscopically, the neoplastic cells were pleomorphic histiocytes with moderate to marked anisocytosis and anisokaryosis. Immunohistochemical staining on cryosections indicated that the neoplastic cells were of myeloid origin, and were positive for CD45 and MHC class II. In addition, there were minor infiltrating cell populations that were positive for B cell markers, CD3, CD4, CD8 and ALV J gp85 antigen. HS in chickens appear to share some morphological and immunohistological features of histiocytic splenic sarcomas in humans and canines. This study provides some novel information on retroviral-induced histiocytic neoplasms in chickens.

Introduction

In the early 1990s, histiocytic proliferative lesions were observed with increasing incidence in broilers condemned in poultry processing plants as “leukosis” and were described as multicentric histiocytosis (MH) (Hafner *et al.*, 1996). MH was reproduced in chickens by inoculating tissue homogenates from chickens with MH but the etiology of MH could not be confirmed since several avian retroviruses including ALVs and reticuloendotheliosis virus (REV) were detected (Goodwin *et al.*, 1999). Similar lesions were reported in the context of subgroup J avian leukosis virus (ALV J) infections from both field and experimental cases and were designated as histiocytic sarcomatosis (Arshad *et al.*, 1997).

In our previous ALV J studies in meat-type chickens at the Avian disease and oncology laboratory (ADOL), in addition to the expected tumors like myelocytomas, nephroblastomas, erythroblastosis and hemangiomas, there was a consistent low incidence (1.1 – 5 %) of histiocytic sarcomas (HS). These tumors were mainly seen in the spleen with occasional to frequent hepatic and renal involvement. The histologic morphology of these tumors is very similar to some histiocytic proliferative lesions in dogs, cats and humans (Spangler and Kass, 1999; Afoller and Moore, 2002). A unique feature of ALV J-induced HS is the minimal viral antigen expression within the tumor cells (Pandiri, 2005). The factors influencing the development of these lesions is poorly understood.

Objective

To evaluate the factors (viral strain and dose, age at infection, host genetics, viral persistence, and host immune response) influencing the pathogenesis of ALV J-induced HS and also to characterize the immunophenotype of the HS lesions.

Materials and methods

Experimental design. Information and samples used in this study were obtained from two experiments aimed at studying different aspects of ALV J persistence (Pandiri, 2005). The effect of strain and dose of ALV J, and age at inoculation on the development of ALV J-induced HS was evaluated retrospectively in experiment #1 involving 374 commercial meat-type chickens inoculated at 5th day of embryonation (*via* Y/S route) or at day of hatch (*via* intra-abdominal route) with either 100 TCID₅₀ or 10,000 TCID₅₀ with one of the three ALV J strains (ADOL Hc1, ADOL 4817, and ADOL 6803) (Fadly and Smith, 1999). Forty five meat-type chickens inoculated with tissue culture medium were used as negative controls.

The effect of chicken strain on the development of ALV J-induced HS was studied retrospectively in experiment #2 involving 75 commercial meat-type chickens and 100 White Leghorn ADOL

Materials and methods (Contd.)

line 0 chickens (Crittenden and Fadly, 1985) infected at hatch (*via* intra-abdominal route) with 1,000 TCID₅₀ of ALV J molecular clone ADOL pR5-4 (Lupiani *et al.*, 2003). Chickens in all the experiments were housed in BL-2 containment in accordance to ADOL animal care and use committee guidelines.

All the chickens were sampled on 6-9 occasions for viremia (V) and neutralizing antibody (A) before the study was terminated at 32 weeks post hatch. The viremia and antibody data from these samplings were classified into 4 infectious profiles *viz.* V+A+, V+A-, V-A+ and V-A-.

At necropsy, tissues with gross lesions were fixed in 10% neutral buffered formalin for routine histologic staining and embedded in Tissue-Tek® O.C.T compound (Sigma Finetek USA, Inc, Torrance, CA) and snap frozen in liquid nitrogen for immunohistochemistry studies.

Virological and serological assays. Virus isolation (VI) and virus neutralization (VN) assays were done as described earlier (Fadly and Witter, 1998; Smith *et al.*, 1979).

Histology. Tissues for histopathology were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections were stained by hematoxylin and eosin (H&E), Giemsa, Snook's reticulin, and Van Gieson's trichrome methods.

Immunohistochemistry. All tissues were stained with a panel of 11 monoclonal antibodies. These include 6-23 (Qin *et al.*, 2001) at 1:500 dilution (ALV J gp85), 5M19 (ChL5) (Barth *et al.*, 1990) at 1:1 dilution (myelomonocytic cells and activated T lymphocytes), K55 (Chung *et al.*, 1991) at 1:10 dilution (common leukocyte antigen CD45), Cla (Ewert *et al.*, 1984) at 1:20 dilution (MHC II), CT3 (Chen *et al.*, 1986) at 1:20 dilution (CD3), CT4 (Chan *et al.*, 1988) at 1:20 dilution (CD4), CT8 (Chan *et al.*, 1988) at 1:1 dilution (CD8), K1 (Chung and Lillehoj, 1991) at 1:5 dilution (macrophages and thrombocytes), CB4 and CB5 (Chen and Cooper, 1987) at 1:25 dilution (B cells), and PAb 240 (Abcam, CA) at 1:25 dilution (p53).

A modified avidin-biotin-peroxidase complex method (Hsu *et al.*, 1981) using the Vectastain® ABC kit (Vector Laboratories; Burlingame, CA) was performed on 5 μm cryosections. Specific brown DAB staining was visualized using a light microscope and the tissues were scored as 0 (no positive cells), 1 (a few scattered positive cells), 2 (moderate number of positive cells), and 3 (large number of positive cells).

Results

Factors influencing the development of ALV J-induced HS lesions (Table 1)

Effect of viral strain, dose and age at infection. There was no effect of strain of ALV J or dose of viral inoculum on the incidence of HS since lesions of varying severity were observed in spleen, liver, and kidney in meat-type chickens inoculated with either 100 TCID₅₀ or 10,000 TCID₅₀ with one of the three ALV J strains (ADOL Hc1, ADOL 4817, and ADOL 6803). All of the HS lesions were only observed in chickens that were inoculated at day of hatch and the incidence of these lesions was 5%. None of the chickens inoculated *in ovo* had evidence of histiocytic proliferative lesions.

Effect of chicken strain. HS lesions were observed only in meat-type chickens but not in White Leghorn ADOL line 0 chickens that were inoculated with ADOL pR5-4 at day of hatch. The incidence in meat-type chickens was 1.3%.

Effect of ALV J infection profile. All the affected chickens had persistent viremia on almost all the sampling intervals and had very little to no NAbs against the inoculated virus. The lesions were observed in chickens that had succumbed to disease starting from 11 weeks post hatch (PH) until the study was terminated at 32 weeks post hatch.

Immunohistochemistry (Table 2, Figure 3). The proliferating tumor cells stained strongly positive with ChL5, K55 and Cla antibodies, variably slightly positive with K1, and negative with CB4/CB5, and p53 antibodies. In addition, there was infiltration of variable proportions of cells staining positive with CT3, CT4, CT8 and 6-23 MAb.

Results (Contd.)

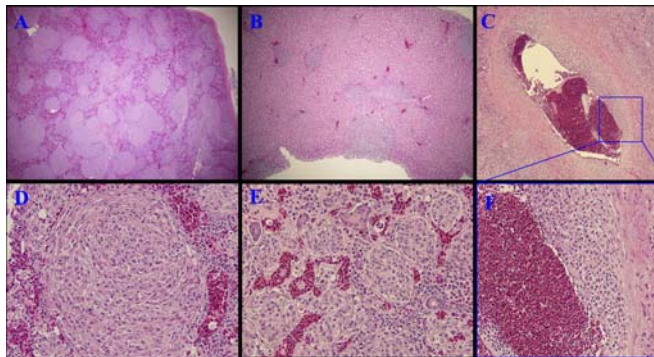


Figure 1. A, D. Multifocal to coalescing histiocytic proliferation within the splenic white pulp (4X); B, E. Multifocal histiocytic proliferations within the hepatic parenchyma; C. Neoplastic thrombi within the hepatic artery at 10X; F. inset 40X of C.

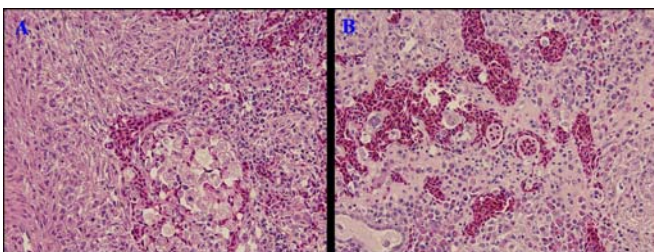


Figure 2. A, B. Splenic tissue sections showing the pleomorphic nature of neoplastic cells. In figure 2A, there is a neoplastic cell population with spindle cell to large foamy epithelioid cell morphology. In addition, there is also mild infiltration of plasma cells. In figure 2B, there is erythrophagocytosis by the neoplastic cells. There is also mild infiltration of plasma cells and myelocytes. Erythrophagocytosis by the neoplastic cells is not a consistent feature though it is observed in some cases. Multinucleated tumor giant cells are not a common feature in any of the HS lesions.

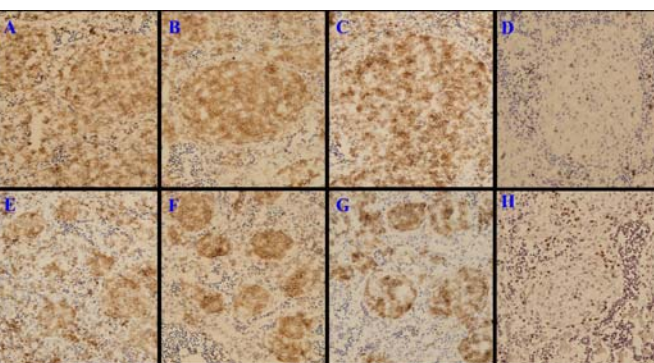


Figure 3. A-F: Immunophenotype of histiocytic proliferations within the spleen (A, B, C) and kidney (D, E, and F). The neoplastic cells within the spleen and liver stain diffusely positive for K55 (A and E), ChL5 (B and F), and Cla (C and G). There is mild infiltration of CD8 (D) and CD4 (H) T cells intermixed within the neoplastic cell population.

Table 1. The effect of age at infection, viral dose, ALV J strain, chicken strain and ALV J infection profile (V and A) on ALV J-induced histiocytic sarcoma

Ch #	Age @ Inoculation	V dose TCID ₅₀ ^a	Viral Strain	Chick Strain	Infection profile V ^b A ^c
1	DOH	100	ADOL Hc1	MT	8/8 0/8
2	DOH	100	ADOL Hc1	MT	7/9 0/9
3	DOH	100	ADOL Hc1	MT	5/5 0/5
4	DOH	10,000	ADOL Hc1	MT	8/8 0/8
5	DOH	10,000	ADOL Hc1	MT	4/8 1/8
6	DOH	10,000	ADOL Hc1	MT	6/6 0/6
7	DOH	10,000	ADOL 6803	MT	9/9 0/9
8	DOH	10,000	ADOL 6803	MT	4/4 1/4
9	DOH	10,000	ADOL 6803	MT	8/9 2/9
10	DOH	100	ADOL 6803	MT	6/7 1/8
11	DOH	100	ADOL 6803	MT	6/6 1/6
12	DOH	100	ADOL 4817	MT	7/7 1/7
13	DOH	100	ADOL 4817	MT	8/8 2/8
14	DOH	100	ADOL 4817	MT	6/7 1/8
15	DOH	1,000	ADOL pR5-4	MT	7/7 2/7

^a Viremia in spleen; ^b viremia in spleen; ^c viremia in spleen. The study was terminated at 32 weeks post hatch. Some chickens have fewer than 9 samplings due to mortality before study termination. Samples stored positive total number of samplings

^d Immunohistochemistry; ^e viremia in spleen; ^f viremia in spleen. Samples tested positive total number of samplings

Table 2. Immunophenotype of the ALV J-induced histiocytic sarcoma tumor cells

Tumor	K55	ChL5	Cla	K1	CT3	CT4	CT8	CB4/5	6-23	p53
HS1	3+	3+	3+	±	1	1	1	-	1	-
HS2	3+	3+	3+	±	1	1	1	-	1	-
HS3	3+	3+	3+	±	1	1	1	-	1	-
HS4	3+	3+	3+	±	1	1	1	-	1	-
HS5	3+	3+	3+	±	1	1	1	-	1	-
HS6	3+	3+	3+	±	1	1	1	-	1	-
ML1	3+	3+	-	-	-	-	-	-	3+	-
ML2	3+	3+	-	-	-	-	-	-	3+	-

Conclusions and discussion

Based on the immunophenotype and histological features, the neoplastic cells are histiocytes of myelomonocytic lineage. The incidence of histiocytic sarcomas in chickens are influenced by host genetics (only meat-type chickens are affected), age at infection (only chickens infected at day of hatch), and ALV J infection profile (persistently viremic chickens with ability to mount an antibody response, albeit an ineffective one).

The histiocytic sarcomas, in this study as well as in other studies, consistently occur in the spleen with frequent involvement of the liver and kidney. HS unlike other ALV J induced tumors occur only in persistently viremic but not tolerized chickens. The pathogenesis of HS seems to be different from other ALV J induced tumors. The viral antigen load within HS is very low compared to other ALV J induced tumors (*data not presented*). It is likely that HS may begin as a reactive process in the spleen against persistent viral load but later progress to neoplasia. The presence of inflammatory cell infiltrate in some tumors supports this hypothesis. The ALV J induced histiocytic proliferative lesions seem to share several histological features with human and canines. These viral-induced histiocytic sarcomas are reproducible consistently and may serve as a comparative model in humans and other animals.

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