Immune Abnormalities in Avian Erythroblastosis Virus-infected Chickens

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ABSTRACT

Infection of animals with retroviruses frequently leads to immunosuppressed states. The immune status of chickens infected with the replication-defective avian erythroblastosis virus (AEV), with its naturally occurring subgroup B helper virus (avian erythroblastosis-associated virus; AEAV), was evaluated daily and compared to the immune status of age-matched uninfected control chickens. Spleen cells from AEV-infected chickens gave depressed responses to concanavalin A, phytohemagglutinin, and pokeweed mitogen beginning 3 days after injection of the virus and continuing until death. Spleen cells from AEV-infected chickens suppressed the T-cell mitogen-induced blastogenic responses of spleen cells from uninfected chickens. The ability of spleen cells from infected chickens to suppress mitogen-induced blastogenic responses of spleen cells from normal chickens in coculture was transient beginning 4 days following viral inoculation, reaching peak levels of suppression on day 7 and disappearing by day 12. Cytolysis of splenic cells from AEV-infected chickens with polyclonal anti-T-cell-serum removed the suppressor activity. Addition of conditioned medium rich in T-cell growth factor resulted in a partial restoration of the blastogenic responsiveness of splenic cells from 6-day post-AEV-infected chickens. Addition of exogenous T-cell growth factor had no effect on the suppressed blastogenic responsiveness of spleen cells from 12-day post-AEV-infected chickens, and it had no effect on coculture suppression. In addition to suppressed T-cell responses to polyclonal mitogen-induced proliferation in vitro and transiently expressed T-suppressor cells, thymic atrophy and structural disruption was observed in AEV-infected chickens.

INTRODUCTION

Infection by avian (1), feline (2), murine (3, 4), and human retroviruses (5, 6) causes either partial or generalized immune suppression. Immune suppression often precedes the development of neoplasms in animals infected by oncogenic retroviruses and persists throughout the tumorigenic process. Immune suppression induced by retroviruses is complex and may involve specifically impaired host immune responses against viral or tumor cell antigens as well as nonspecific immunosuppression. The virus itself or virus-related proteins may be immunosuppressive, host immunoresponsive cells may be inactivated or eliminated by the virus, or host immunosuppressive cells or products may be induced (1, 7, 8).

Although the cascade of events leading to immune suppression in retrovirus infections is not fully characterized there is evidence that certain retrovirus proteins, especially proteins related to the retrovirus envelope-encoded 15,000-dalton molecule, referred to as P15E-related proteins, presumably synthesized by infected cells, may represent a major contributing immunosuppressive factor (3, 4, 9–11). The capacity to induce immune suppression has been observed among members of the four major groups of avian retroviruses: the REVs (the transforming virus, REV-T, and the associated helper virus, REV-A) and the avian sarcoma-leukosis complex (which includes AEV), avian myeloblastosis virus, and avian myelocytomatosis virus (MC-29), and their associated helper viruses AEAV, MAVE, and myelocytomatosis-associated virus, respectively). Immunosuppression has been systematically studied in only two of the avian retrovirus systems, REV (12–15) and MAV-2(0) (16–18).

The C-type retrovirus, AEV, causes a fatal erythroblastic anemia within 2–3 weeks after infection (19, 20). Other than suppression of the PHA-induced blastogenic response of spleen cells from AEV-infected chickens (1), the status of the immune response system during the preleukemic and leukemic phases of AEV-induced erythroleukemia has not been reported. In the present study, daily evaluation of the mitogen-induced blastogenic responses of spleen cells from AEV-infected chickens was conducted in an attempt to gain a better understanding of AEV-induced immune dysfunctions. These studies: (a) showed a generalized suppression of splenic T-cell mitogen-induced blastogenic responses that were partially reversible by addition of TCGF, (b) identified transiently expressed T-suppressor cell activity in the spleen of infected chickens that was not influenced by addition of exogenous TCGF, and (c) identified thymus dysfunctions characterized by extreme thymic atrophy, depletion of medullary thymocytes, and disruption of connective tissue separating the thymic lobules.

MATERIALS AND METHODS

Viruses and Experimental Animals. The Hyline International SC strain of B/B' chickens, purchased as fertile eggs from Hyline International (Johnston, IA), were challenged with virus at 13-day posthatch and sacrificed at daily intervals following virus inoculation. Uninfected age-matched controls and infected chickens were housed in separate cages throughout the experiments. AEV-R [a replication-defective avian erythroblastosis virus, with its naturally occurring associated subgroup B replication-competent helper virus (AEAV) (21)] was utilized throughout these studies. Chickens were virally challenged by injecting approximately 2.5 x 10^6 focus forming units i.v. (20). Peripheral blood samples were taken prior to sacrifice and evaluated for the presence of erythroblasts and percentage of packed RBC (22).

Source and Preparation of Lymphocytes. Cells were flushed from individual spleens of AEV-inoculated chickens, age-matched uninfected chickens, and normal adult chickens by gentle teasing of the spleens after decapsulation while flushing with serum-free RPMI 1640 (Irvine Scientific, Santa Anna, CA). Splenic cells were washed three times in the same media and numbers of viable cells were determined by trypan blue exclusion assays. The required cell concentrations were made up in serum-free RPMI 1640 supplemented with penicillin (100 IU/ml; GibCO, Grand Island, NY), streptomycin (100 μg/ml; GibCO) and glutamine (200 mM; Sigma Chemical Co., St. Louis, MO), hereafter referred to as supplemented media.

Assay for Mitogen Responsiveness. PHA-P (Difco, Detroit, MI), Con A (Grade III; Sigma), and PWM (9379; Sigma) were used in a well-established serum-free mitogen assay system (23). Aliquots (0.2 ml) of

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3The abbreviations used: REV, reticuloendotheliosis virus; AEV, avian erythroblastosis virus; AEAV, avian erythroblastosis-associated virus; Con A, concanavalin A; NRS, normal rabbit serum; PHA, phytohemagglutinin; PWM, pokeweed mitogen; TCGF, T-cell growth factor; MAVE, myeloblastosis-associated virus; CM, conditioned media.
cultures were then incubated in a humidified atmosphere of 5% carbon dioxide in air at 37°C for 72 h. Forty-eight h after initiation of coculture assay described above. The adherent cell fraction was flushed by gentle rotation of the microtiter plate (Costar 3596). Predetermined optimal concentrations of PHA (0.5 μl/0.05-ml aliquot/culture), Con A (0.5 μg/0.05-ml aliquot/culture), PWM (0.5 μg/0.05-ml aliquot/culture), or no mitogen for controls were then added to duplicate or triplicate cultures. Cell cultures were then incubated in a humidified atmosphere of 5% carbon dioxide in air at 37°C for 72 h. Forty-eight h after initiation of incubation, 0.5 μCi of tritiated thymidine ([3H]thymidine, 55 Ci/mmol; New England Nuclear, Boston, MA) was added per culture. After an 18-h pulse, the cells were washed with phosphate-buffered saline (pH 7.2) and harvested onto glass fiber filters (Whatman Reeve Angel, Clifton, NJ) with an automatic Mash II Cell Harvester (Microbiological Associates, Walkersville, MD). Air dried filters were immersed in 2 ml of Aquasol (New England Nuclear) and radioactivity was counted in an LKB RackBeta liquid scintillation counter. Results are expressed as [3H]thymidine uptake in counts per minute (cpm).

Assay for Presence ofSuppressor Activity. To test for the presence of suppressor cells, spleen cells from AEV-infected chickens were cocultured with spleen cells from normal adult chickens, and mitogen-stimulated blastogenesis analyzed. Coculture analyses involved incubating spleen cells from normal chickens at 6 × 10⁶ cells/culture with spleen cells from AEV-infected chickens or age-matched, uninfected chickens at 6 × 10⁶/culture, 4 × 10⁵/culture, and 2 × 10⁵/culture. The assay for determining responsiveness to mitogens in coculture was performed as described above.

Depletion of Specific Spleen Cells by Treatment with Anti-T or Anti-B Sera. Rabbit anti-chicken thymocyte serum (Microbiological Assoc. Inc.) and anti-chicken B-cell serum (prepared in White New Zealand rabbits against bursal-derived lymphocytes from 1-day posthatch SC chickens), were made T- and B-cell specific by repeated adsorptions with bursal or thymus cells, respectively. These antisera were demonstrated to be B- and T-cell specific by immune fluorescence, and by their ability to specifically lyse bursa and thymus cells, respectively (24). These antisera were used in a complement-mediated lysis assay to deplete B- and T-lymphocytes from spleen cell preparations derived from AEV-inoculated chickens (24). These selectively depleted spleen cell preparations were then examined for their ability to suppress normal spleen cell proliferative responses to mitogens in coculture (24). Briefly, spleen cells from AEV-infected and uninfected normal chickens were incubated with anti-T serum, anti-B serum, or normal rabbit serum for 30 min at 25°C. Guinea pig complement (GIBCO) that had been adsorbed with normal chicken spleen and peripheral RBC at 4°C for 30 min immediately prior to use, was added at a final dilution of 1:20. The reaction mixture was incubated at 37°C with gentle rotation for 30 min. The reaction was stopped by washing the treated cells three times in RPMI 1640. The number of viable cells remaining after treatment was determined by trypan blue exclusion analyses. The treated cells were resuspended in supplemented media back to their original volume and assayed in coculture for their ability to suppress mitogen-induced proliferative responses.

Assay for Suppressive Activity of Adherent and Nonadherent Cell Fractions. Spleen cell preparations (2.0 ml) were diluted to 3 or 6 × 10⁶ viable lymphocytes per milliliter of RPMI 1640 medium containing 5% fetal calf serum (MA Bioproducts, Walkersville, MD). The spleen cell preparations were incubated for 2 h at 37°C in 60-mm disposable plastic petri dishes. Following incubation, nonadherent cells were carefully decanted and washed twice in RPMI 1640 and used in the mitogen coculture assay described above. The adherent cell fraction was flushed from the petri dishes, washed twice, and assayed for ability to suppress mitogen responses in coculture. Morphology of plastic-adherent cells was determined by microscopic examination of cells stained with Wright's stain, using criteria of Lucas and Jamroz (25).

Production and Bioassay for TCGF. The production and biological functional assay of chicken TCGF was performed using previously described procedures (26). Briefly, CM was obtained from spleen cell cultures (1 × 10⁶/ml) which had been stimulated by incubation with a Con A-erythrocyte complex (300 μg of Con A/6 × 10⁶ chicken erythrocytes/ml) for 24 h. The CM was dialyzed against RPMI 1640 culture medium for 48 h at 4°C using 10-mm dialysis tubing. The CM was then stored frozen in 1-ml aliquots at −20°C until immediately prior to use. TCGF activity of the CM was determined by thymidine uptake using a bioassay in which T-cell blasts, isolated from spleen cells obtained from an adult chicken on a discontinuous Percoll (Pharmacia) gradient, were incubated together with 5, 10, 15, 20, 25, 30, and 40% TCGF. Non-Con A-erythrocyte complex-stimulated culture media served as the control (data not shown). The ability of TCGF to enhance AEV-suppressed spleen cell proliferation was determined by culturing spleen cells from AEV-infected chickens (separately and in coculture) in the presence of 5, 10, 15, 20, 25, and 30% TCGF.

Histological Examination of Thymus and Bursa. Thymus and bursa size and morphology were examined at daily intervals following AEV inoculation. Age- and sex-matched uninfected chickens served as controls. Prior to sacrifice, whole body weights were obtained, and the chickens were bled for analyses of erythroblasts in the peripheral blood and percentage of packed RBC. Following sacrifice, the bursa and thymus lobes were carefully removed, trimmed, weighed, and fixed in 10% phosphate-buffered formalin. The organs were embedded in paraffin, sectioned at 5–6 μm, mounted, and stained with Harris' hematoxylin and eosin (Sigma). The stained slides were examined using light microscopy and photographed at ×10 and ×40 using Panatomic-X film (ASA 32).

RESULTS

Mitogen-induced Proliferation of Spleen Cells Obtained from AEV-infected and Uninfected Chickens. Using optimal culture conditions for normal chicken spleen cells (taking into account cell density, mitogen concentration, [3H]thymidine uptake kinetics, and cell viability), spleen cells from AEV-infected chickens sacrificed at varying time intervals following AEV inoculation exhibited suppressed responses to Con A, PHA, and PWM stimulation (Table 1). Suppressed mitogen-induced responsiveness by spleen cells from AEV-infected chickens was evident 4 days following AEV inoculation with more pronounced suppression being evident on the 5th and succeeding days following AEV inoculation. Inability of spleen cells from AEV-infected chickens to respond to Con A, PHA, and PWM remained until death of the chickens at approximately 14 days following AEV inoculation. The suppressed mitogen-induced blastogenic responses of spleen cells obtained from AEV-infected chickens was not affected by mitogen concentrations (PHA at 0.1–1.0 μl/culture; Con A and PWM at 0.1–1.0 μg/ml) or spleen cell concentrations ranging from 0.5 to 6.0 × 10⁶ (data not shown).

Ability of Spleen Cells from AEV-infected Chickens to Suppress Mitogen-induced Proliferation of Spleen Cells from Normal Adult Chickens. The Con A, PHA, and PWM mitogen-induced responses of spleen cells from normal adult chickens were markedly suppressed when cocultured with spleen cells from AEV-infected chickens at a ratio of 1:1 (Table 2). The ability of spleen cells from AEV-infected chickens to suppress mitogen-induced proliferation of spleen cells from normal chickens was evident 4 days following AEV inoculation; however, the suppression was transient with the highest degree of suppression occurring 7 days following AEV inoculation.

Identification of Transiently Expressed Splenic T-suppressor Cell Activity in AEV-infected Chickens. The ability of spleen cells from AEV-infected chickens to suppress in coculture following the selective removal of T- and B-lymphocytes by antibody complement-mediated cytolysis and removal of plastic adherent cells is depicted in Fig. 1. Pretreatment of spleen cells from 6-day post-AEV-infected chickens with anti-T serum and complement before coculturing with spleen cells from normal adult chickens greatly reduced the ability of spleen cells from the AEV-infected chickens to suppress Con A and PHA mito-
gen-mediated blastogenic responses in coculture, indicating that the suppressor cell activity was associated with T-cells. In contrast, treatment of spleen cells from AEV-infected chickens with anti-B serum or normal rabbit serum plus complement, or removal of plastic-adherent cells had little effect on the ability of the remaining cell population to suppress Con A- and PHA-induced proliferation of spleen cells from normal adult chickens.

The Ability of TCGF to Partially Restore the Suppressed Host Spleen Cell Mitogen Response and the Inability of TCGF to Affect T-Cell Suppression in Coculture. The suppressed responses of spleen cells from 6-day post-AEV-infected chickens to PHA, Con A, and PWM mitogen stimulation could be partially ameliorated by the addition of exogenous TCGF (Table 3). ConA-, PHA-, and PWM-induced proliferation was enhanced by 72, 54, and 52%, respectively, when the spleen cells were treated with optimal concentrations (25%) of TCGF. However, the mitogen-induced blastogenic response of spleen cells from AEV-infected chickens could not be restored, by the addition of TCGF, to the same level of mitogen responsiveness of spleen cells obtained from uninfected age-matched controls. The addition of TCGF to spleen cells from AEV-infected chickens 12 days post-AEV infection was not capable of restoring mitogen-induced proliferation (data not shown). Addition of TCGF to cocultures containing spleen cells from AEV-infected chickens

Table 1 Mitogen-induced proliferation of spleen cells from AEV-infected and uninfected age-matched control chickens

<table>
<thead>
<tr>
<th>Days following AEV inoculation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Treatment</th>
<th>+ Con A&lt;sup&gt;b&lt;/sup&gt; (0.5 μg/culture)</th>
<th>% of suppression&lt;sup&gt;c&lt;/sup&gt;</th>
<th>+ PHA&lt;sup&gt;b&lt;/sup&gt; (0.5 μg/culture)</th>
<th>% of suppression&lt;sup&gt;c&lt;/sup&gt;</th>
<th>+ PWM&lt;sup&gt;b&lt;/sup&gt; (0.5 μg/culture)</th>
<th>% of suppression&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Infected</td>
<td>54,996 ± 1,527&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5</td>
<td>73,030 ± 6,673</td>
<td>5</td>
<td>21,804 ± 1,020</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>57,743 ± 7,820</td>
<td></td>
<td>76,680 ± 1,838</td>
<td></td>
<td>18,082 ± 2,429</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Infected</td>
<td>65,875 ± 4,811</td>
<td>28</td>
<td>59,209 ± 9,975</td>
<td>28</td>
<td>12,283 ± 695</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>91,913 ± 1,210</td>
<td></td>
<td>82,082 ± 4,693</td>
<td></td>
<td>20,354 ± 4,737</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Infected</td>
<td>15,066 ± 6,525</td>
<td>59</td>
<td>16,923 ± 2,447</td>
<td>49</td>
<td>7,883 ± 265</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>36,410 ± 1,075</td>
<td></td>
<td>32,900 ± 4,288</td>
<td></td>
<td>10,818 ± 831</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Infected</td>
<td>11,702 ± 2,791</td>
<td>74</td>
<td>15,562 ± 3,617</td>
<td>75</td>
<td>3,970 ± 589</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>44,177 ± 407</td>
<td></td>
<td>62,474 ± 4,669</td>
<td></td>
<td>10,424 ± 2,403</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Infected</td>
<td>13,234 ± 3,104</td>
<td>56</td>
<td>7,085 ± 117</td>
<td>82</td>
<td>1,089 ± 259</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>30,348 ± 367</td>
<td></td>
<td>38,903 ± 5,763</td>
<td></td>
<td>8,063 ± 494</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Infected</td>
<td>16,092 ± 2,099</td>
<td>61</td>
<td>4,568 ± 2,259</td>
<td>92</td>
<td>983 ± 73</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>41,568 ± 3,073</td>
<td></td>
<td>56,538 ± 2,091</td>
<td></td>
<td>14,151 ± 518</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Infected</td>
<td>10,405 ± 3,864</td>
<td>82</td>
<td>3,649 ± 2,103</td>
<td>92</td>
<td>276 ± 142</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>57,448 ± 1,442</td>
<td></td>
<td>46,953 ± 2,505</td>
<td></td>
<td>17,181 ± 1,663</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Spleen cells were cultured at 1.2 × 10<sup>6</sup> cells/culture.
<sup>b</sup> Chickens were inoculated with AEV (AEAV) at 13 days post-hatch.
<sup>c</sup> Percentage of suppression of normal response was determined by dividing the mean cpm of infected by the mean cpm of uninfected and subtracting from 100.
<sup>d</sup> Average [3H]thymidine uptake (minus background) in cpm ± S.D. of triplicate replicas from four to eight individual chickens. Background (spleen cells cultured in the absence of mitogens) responses were low, ranging between 25 and 400 cpm.

Table 2 Effects of coculturing<sup>a</sup> spleen cells from AEV-infected or age-matched uninfected chickens on the Con A, PHA, and PWM responses of normal adult chicken spleen cells

<table>
<thead>
<tr>
<th>Days following AEV inoculation&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Treatment</th>
<th>+ Con A&lt;sup&gt;c&lt;/sup&gt; (0.5 μg/culture)</th>
<th>% of suppression&lt;sup&gt;d&lt;/sup&gt;</th>
<th>+ PHA&lt;sup&gt;c&lt;/sup&gt; (0.5 μg/culture)</th>
<th>% of suppression&lt;sup&gt;d&lt;/sup&gt;</th>
<th>+ PWM&lt;sup&gt;c&lt;/sup&gt; (0.5 μg/culture)</th>
<th>% of suppression&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Infected</td>
<td>67,044 ± 4,411</td>
<td>1</td>
<td>74,123 ± 2,820</td>
<td>10</td>
<td>24,191 ± 585</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>67,888 ± 864</td>
<td></td>
<td>82,416 ± 8,517</td>
<td></td>
<td>25,517 ± 1,555</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Infected</td>
<td>74,148 ± 2,723</td>
<td>23</td>
<td>69,915 ± 5,374</td>
<td>26</td>
<td>27,003 ± 761</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>95,685 ± 2,620</td>
<td></td>
<td>94,771 ± 1,572</td>
<td></td>
<td>28,707 ± 3,051</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Infected</td>
<td>27,693 ± 5,804</td>
<td>49</td>
<td>30,151 ± 4,610</td>
<td>38</td>
<td>4,900 ± 968</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>54,805 ± 5,960</td>
<td></td>
<td>48,485 ± 1,945</td>
<td></td>
<td>26,445 ± 400</td>
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</tr>
<tr>
<td>6</td>
<td>Infected</td>
<td>30,170 ± 4,135</td>
<td>64</td>
<td>38,631 ± 5,839</td>
<td>47</td>
<td>6,558 ± 1,441</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>82,688 ± 4,900</td>
<td></td>
<td>72,573 ± 310</td>
<td></td>
<td>17,086 ± 16</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Infected</td>
<td>11,851 ± 1,768</td>
<td>77</td>
<td>8,341 ± 546</td>
<td>84</td>
<td>4,713 ± 274</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>51,277 ± 3,577</td>
<td></td>
<td>50,535 ± 4,131</td>
<td></td>
<td>14,344 ± 2,432</td>
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</tr>
<tr>
<td>8</td>
<td>Infected</td>
<td>41,670 ± 8,374</td>
<td>37</td>
<td>31,352 ± 5,476</td>
<td>34</td>
<td>7,026 ± 392</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>65,901 ± 2,504</td>
<td></td>
<td>47,264 ± 2,918</td>
<td></td>
<td>16,105 ± 1,142</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Infected</td>
<td>43,236 ± 9,198</td>
<td>19</td>
<td>45,936 ± 4,011</td>
<td>10</td>
<td>23,103 ± 1,366</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Uninfected</td>
<td>53,027 ± 4,248</td>
<td></td>
<td>50,569 ± 6,433</td>
<td></td>
<td>22,248 ± 425</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Cocultures (1:1) were conducted in which 6 × 10<sup>6</sup> spleen cells from a normal adult chicken were mixed with 6 × 10<sup>6</sup> spleen cells from either an AEV-infected chicken or from an age-matched uninfected chicken per culture.
<sup>b</sup> All chickens used in these studies were inoculated with AEV (AEAV) at 13 days posthatch.
<sup>c</sup> Percentage of suppression of normal response:

\[
100\% - \frac{\text{Mean cpm of coculture of normal adult spleen cells with spleen cells from infected chickens}}{\text{Mean cpm of coculture of normal adult spleen cells with spleen cells from appropriate age-matched uninfected chickens}} \times 100
\]

<sup>d</sup> Average [3H]thymidine uptake in cpm ± S.D. of duplicate cultures from four to eight individual chickens. Background counts ranged from 25 to 400 cpm for cells cultured in the absence of mitogens and were subtracted prior to determining average thymidine uptake.
Immune Abnormalities in AEV-infected Chickens

Fig. 1. Spleen cells from 6-day post-AEV-inoculated chickens (note: chickens were infected at 13 days of age) and uninfected, age-matched controls were aliquoted. Individual aliquots were depleted of specific cell populations by either complement-mediated cytolysis with antisera specific for T- and B-cells, or depleted of plastic-adherent cells, or left untreated. Spleen cell preparations depleted of B-lymphocytes, T-lymphocytes, and adherent cells were examined for ability to suppress mitogen-induced blastogenic responses of spleen cells from normal adult chickens when examined in a standard coculture mitogen assay. Spleen cell populations treated with normal rabbit serum plus complement (NRS + C) served as controls. Data are expressed as the mean tritiated thymidine uptake ± S.D. of triplicate cultures from three experiments. Normal adult chicken spleen cells cultured alone gave responses of 49.022 ± 6.255 and 49.548 ± 294 to Con A and PHA stimulation, respectively. Background proliferation of cultured cells in the absence of mitogens ranged from 218 to 865 cpm.

Table 3 Effects of T-cell growth factor (TCGF) on mitogen-induced proliferation of spleen cells from AEV-infected and uninfected age-matched control chickens

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mitogen</th>
<th>TCFG</th>
<th>+TCGF</th>
<th>% of increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenic lymphocytes from 6-day post-AEV inoculation</td>
<td>Con A</td>
<td>3.686 ± 1.77</td>
<td>12.980 ± 7.000</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>5.048 ± 1.166</td>
<td>11.036 ± 3.268</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>PWM</td>
<td>2.789 ± 2.066</td>
<td>5.845 ± 1.581</td>
<td>52</td>
</tr>
<tr>
<td>Spleen cells from uninfected age-matched controls</td>
<td>Con A</td>
<td>34.869 ± 1.084</td>
<td>35.657 ± 479</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>52.480 ± 3.432</td>
<td>49.333 ± 3.131</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PWM</td>
<td>12.387 ± 2.017</td>
<td>12.963 ± 309</td>
<td>4</td>
</tr>
<tr>
<td>Coculture analyses of spleen cells taken from 6-day post-AEV inoculated chickens</td>
<td>Con A</td>
<td>39.825 ± 6.542</td>
<td>39.156 ± 7.228</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>39.912 ± 9.374</td>
<td>38.761 ± 11.672</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PWM</td>
<td>12.827 ± 1.843</td>
<td>11.516 ± 4.129</td>
<td>0</td>
</tr>
<tr>
<td>Coculture analyses of spleen cells taken from uninfected age-matched control chickens</td>
<td>Con A</td>
<td>98.202 ± 6.328</td>
<td>96.775 ± 4.330</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>103.767 ± 5.847</td>
<td>98.864 ± 5.471</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PWM</td>
<td>41.017 ± 3.326</td>
<td>39.672 ± 3.468</td>
<td>0</td>
</tr>
</tbody>
</table>

6 days post-AEV infection and normal adult chickens did not affect the suppressed Con A- and PHA-mediated blastogenic responses (Table 3).

AEV-induced Thymus Abnormalities. Thymus indices (weights of thymus lobes divided by total body weight) obtained from AEV-infected chickens showed a normal profile up to 6 days post-AEV infection (Fig. 2). On the 8th day following AEV inoculation the thymus index was slightly higher than the thymus index of uninfected age-matched controls. However, from 8 days post-AEV infection until death there was a progressive decrease in the size of the thymus lobes. Thymus lobes obtained from AEV-infected chickens at 16 days postinoculation were extremely small. Analysis of thymus lobes from 12 AEV-infected chickens with hematocrits of 17.1% (age-matched controls exhibited hematocrits of 36%), showed a thymus weight of 0.21 ± 0.05 g compared to thymus lobes of age-matched, uninfected controls which weighed 1.65 ± 0.47 g. In addition to thymic anomalies, the spleens and livers of AEV-infected chickens were enlarged [spleenic index of 0.470 ± 0.20 (AEV) and 0.230 ± 0.04 (control); and hepatic index of 4.40 ± 0.30 (AEV) and 3.30 ± 0.60 (control)], however, neither spleens nor livers exhibited any tumor nodules or necrotic lesions. The size of bursas obtained from AEV-infected chickens do not differ significantly from the size of bursas obtained from age-matched uninfected controls (12-day post-AEV bursal index = 0.570 ± 0.09; age-matched control bursal index = 0.60 ± 0.040). Furthermore, histological analyses show the bursas of AEV-infected chickens to exhibit structural integrity similar to uninfected controls (data not shown).

Histological comparison of thymus lobes from AEV-infected and age-matched uninfected chickens (Fig. 3, A-D) showed that AEV infection caused thymic abnormalities. Thymus lobes were atrophied and exhibited structural disruption with infiltration of loose interlobular connective tissue resulting in collapse and fusion of the medullary portion of the different lobules. The thymic medulla appeared to be depleted of lymphocytes and infiltrated with large reticular cells.

Discussion

A generalized immune suppression prior to neoplasia is common for most neoplasms induced by oncogenic retroviruses (8, 27–29). AEV is an acute replication-defective retrovirus that, in conjunction with its replication-competent associated subgroup B-helper virus, AEAV, causes a fatal erythroleukemia.
Immune abnormalities in AEV-infected chickens appear to occur in three distinct stages. Stage 1 occurs 3–4 days following viral inoculation and is characterized by two immune dysfunctions. One immune dysfunction is manifested by the relative inability of spleen cells to be induced to proliferate in response to mitogen stimulation. The inability to respond increases progressively following AEV inoculation and the mitogen responses (*i.e.*, Con A, PHA, and PWM) remain suppressed until death. The second immune dysfunction is the induction of host-derived splenic T-suppressor cell activity. The T-suppressor cell activity is transient, reaching peak suppressive levels by 7 days post-AEV inoculation and disappearing by 10–12 days post-AEV inoculation. Stage 2 of tumorigenesis is characterized by the onset of a rapid, severe anemia, rapid weight loss and thymic atrophy beginning approximately 8 days following AEV inoculation. Stage 3 is characterized by the influx of erythroblasts into the peripheral blood, usually around 12–14 days post-AEV inoculation, followed by death in 2–3 days.

The capacity to suppress T-cell mitogen responses has been observed with acute leukemia viruses (myelocytomatisos virus MC-29, AEV, AMV, and REV (reviewed in Refs. 8 and 18). These investigations show that the immunosuppression is associated with helper viruses present in stocks of the acutely oncogenic components.

The AEV-induced abnormalities reported in this paper involved chickens infected with both AEV and AEAV, and therefore, did not address the separate contributions of the transforming and helper viruses to the induced immune abnormalities. However, studies in this laboratory have shown that suppressed mitogen responses and T-suppressor cell activity occur in chickens injected with UV-irradiated AEV and AEAV, demonstrating that the suppressed immune states can be achieved in the absence of virally transformed tumor cells. These observations suggest that a virion component is responsible for immune suppression by AEV.

Immune abnormalities induced by AEV, REV, and MAV-2(0) are similar, yet distinct (8, 18, 32, 33). In all three cases mitogen-induced proliferation of spleen cells from uninfected chickens are suppressed when cocultured with spleen cells from infected chickens. In AEV and REV infections suppressed coculture mitogen responses involve a transiently expressed T-cell suppressor activity (8), while in MAV-2(0) infections, suppression results from an interference of accessory function(s) of macrophage-like adherent cells (16–18). The addition of exogenous chicken TCGF, that is biologically and biochemically similar to mammalian interleukin 2 (34, 35) or the addition of adherent cell populations obtained from the spleens of uninfected chickens, reversed the suppression induced by MAV-2(0) (17, 36) suggesting that MAV-2(0) affects macrophage function resulting in disrupted TCGF production by helper T-cells (36).

Studies reported in this paper indicate that suppressed T-cell mitogen responses in AEV-infected chickens can be partially reversed by the addition of TCGF during the early stages of infection (6 days post-AEV inoculation) but not later, *i.e.*, 12 days post-AEV inoculation. Addition of optimal amounts of exogenous TCGF to spleen cells from AEV-infected chickens only partially restored the ability of splenic lymphocytes to respond to Con A and PHA, perhaps indicating the involvement of some factor other than inadequate levels of TCGF in AEV-induced immune suppression. The inability of TCGF to affect the suppression of blastogenic responses of splenic lymphocytes from 12-day post-AEV-inoculated chickens suggests that the T-cells are refractory to TCGF or that the responsive cells have been destroyed. The gradual depletion of lymphocytes in the thymus of AEV-infected chickens supports the latter possibility. The inability of exogenous TCGF to affect the coculture suppression suggests that the AEV-induced T-suppressor cell activity is TCGF independent, and further suggests that the AEV-induced suppressed splenic responses and the T-suppressor cell activity involve different mechanisms.

Thymus atrophy with accompanying collapse of the thymus structure starting at approximately 8 days following viral inoculation was documented by analyzing thymic weights in relation to total body weight and by examination of histologically stained thymus tissue sections. Thymus lesions in AEV-infected chickens were similar to thymic lesions reported with feline leukemia virus (37) and murine leukemia virus (38, 39), being characterized by depleted cortical thymocytes, infiltration with large reticular cells, and the disruption of interlobular connective tissue leading to collapse and fusion of the different lobes. The size and structural integrity of bursas obtained from 12-day post-AEV-inoculated chickens do not differ significantly from the bursas obtained from age-matched uninfected controls.

*Fig. 2. Thymus indices were determined for AEV-infected and uninfected chickens. Thymus lobes from age- and sex-matched AEV-infected and uninfected chickens (body weight determined at time of sacrifice) were removed, stripped of fat, and weighed. The data are depicted as mean thymus indices ±S.D.*

Data are derived from analyses of five chickens/treatment group at each day of sacrifice.
Immune abnormalities in AEV-infected chickens

Fig. 3. Thymus lobes were removed from 16-day post-AEV-infected and uninfected age-matched control chickens, fixed in 10% phosphate-buffered formalin, embedded in paraffin, and sectioned at 5–6 μm. The sections of thymus tissue were stained with Harris’ hematoxylin and eosin and examined with light microscopy. Photographs were taken at ×10 (A, uninfected; B, AEV-infected) and ×40 (C, uninfected; D, AEV-infected).

Infection of chickens with REV causes disruption of the thymus and bursa (32), while infection with MAV-2(0) does not appear to affect either the bursa or thymus when chickens are infected after hatching (40). However, when MAV-2(0) is used to infect chick embryos there is a profound affect on both the thymus and bursa (41).

The suppressed T-cell mitogen responses, the inability of exogenous addition of TCGF to correct the defect, and the disruption and atrophy of the thymus during the final stages of AEV infection suggest that T-lymphocytes are being disrupted, destroyed, or suppressed in production. Studies are in progress to test these possibilities.

Although the cascade of events leading to immune dysfunctions in retrovirus infections are not fully characterized, there is evidence that certain retrovirus proteins may be involved (3, 4, 9, 11). The virally coded M, 15,000 transmembrane envelope protein of the feline leukemia virus, referred to as p15E, has been shown to play a major role in the induction of immunosuppression in vivo (10, 11), induction of thymus atrophy and accompanying lesions (37), and has been shown to inhibit lymphocyte blastogenesis in vitro (3, 9, 42, 43). p15E-related molecules appear to be a common factor among many retroviruses with extensive homology occurring between diverse retroviral transmembrane proteins (28). Studies are in progress to determine if p15E-like molecules are responsible for the immune dysfunctions observed in AEV-infected chickens.

Acknowledgments

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References

IMMUNE ABNORMALITIES IN AEV-INFECTED CHICKENS


Immune Abnormalities in Avian Erythroblastosis Virus-infected Chickens

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