Tubuloreticular structures in chicken bursa Fabricii lymphocytes

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Summary. Tubuloreticular structures were observed in bursal lymphocytes of a 12-day-old male chicken. These structures are often observed in mammalian lymphocytes and endothelial cells. Their presence in bursal lymphocytes confirm their association with B-lymphocytes.

Tubuloreticular structures (TRS) are systems of anastomosing tubules found in the endoplasmic reticulum of a number of cells of normal and pathological tissues[2-4]. They occur most frequently in lymphocytes and endothelial cells of mammals[4]. Their role is still unknown and possibly they perform different functions in different cells[3,5-7]. To our knowledge they have not previously been reported in birds.

1 bursa of Fabricius of a 12-day-old male chicken from a commercial strain (Hubbard), afflicted with an undiagnosed disease, was studied by electron microscopy. Further work on bursas of older birds similarly affected, disclosed the presence of C-type oncornavirus particles, a finding suggestive of leukosis[8]. The chicken was vaccinated against Newcastle disease. Fragments of less than 1 mm^3 of the bursa were fixed sequentially in 3% glutaraldehyde in cacodilate buffer pH 7.3, 1% osmium tetroxide in the same buffer and 0.5% uranyl acetate in bi-distilled water. Following dehydration in ethanol, they were embedded in an epon-araldite mixture. Pale gold to silver sections were contrasted with lead cytrate (Reynolds) and observed in an electron microscope JEOL 100C.

Tubuloreticular structures were found inside endoplasmic reticulum cisternae of bursal lymphocytes (figure 2). They are formed by closely packed arrays of dense, branched structures (figures 1 and 2), measuring 20–25 nm in cross section. A few suggestions of a tubular nature, and continuity with the endoplasmic recticulum membranes can be observed (figures 1 and 2). No viral particles were found in this chicken.

Tubuloreticular structures are frequently found in mammals associated with viral, autoimmune and neoplasic diseases, and sometimes in normal tissues also[4]. They have not so far been detected in birds.

The chicken studied was vaccinated against Newcastle disease. TRS are often thought to represent nucleocapsids of paramixoviruses[9,10]. However, cytochemical evidence has failed to confirm this hypothesis[3,5,7], and it is clear from critical ultrastructural studies that they differ in important morphological aspects from viral nucleocapsids[5,11]. The structures observed in the present study are similar to those previously reported in mammals, particularly to those with more tightly packed tubules[12], which are seen preferentially

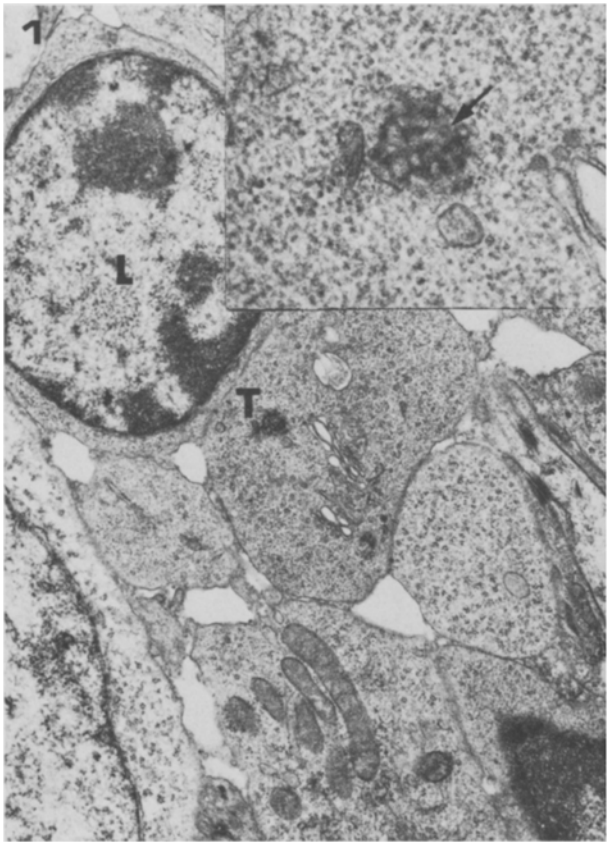

Fig. 1. Low magnification of bursal lymphocytes (L). A tubuloreticular structure (T) can be seen near a dictiosome. × 13,700. Inset: High magnification of the same structure. Circular profile (arrow) suggests tubular nature of the dense strands. × 55,000.

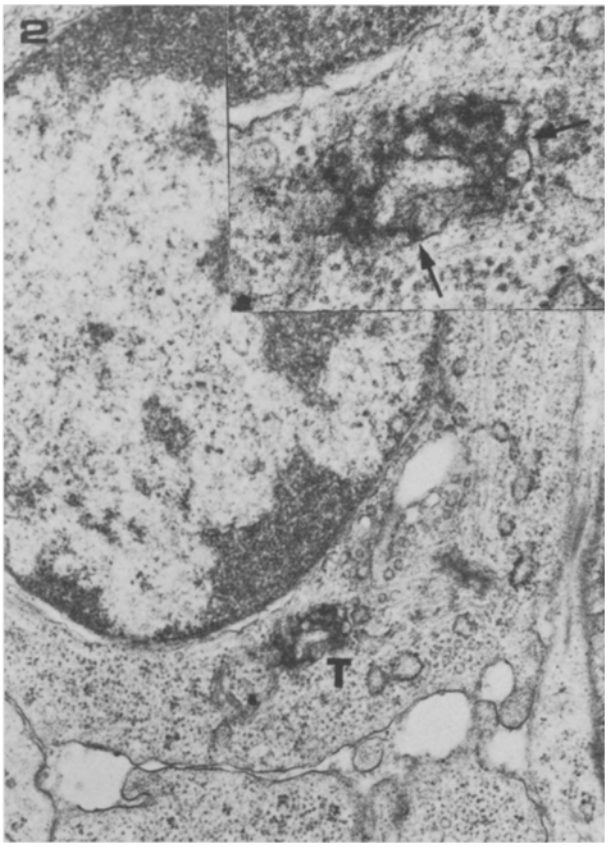

Fig. 2. Tubuloreticular structure (T) can be seen inside a smooth endoplasmic reticulum cisterna. × 26,400.
Inset: Enlargement of TRS shows apparent continuities (arrows) of the tubules with the cisternal membrane. × 77,000.

in neoplasic conditions and virus infections[11]. We consider it therefore unlikely that they represent some kind of expression of the vaccinal virus, but a relationship to virus replication cannot be dismissed on the basis of available data.

The presence of TRS in bursal lymphocytes is interesting in view of the observations of L. Pothier et al.[6] which found a correlation of their occurrence with the synthesis of IgG in a series of transplantable tumours of human lymphoid origin. Also, the induction of TRS by 5-bromo-2'-deoxyuridine[13] seems to succeed primarily in Epstein Barr virus-positive B-cell lines[14]. These observations point to an association of TRS with B-lymphocytes.

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In vitro cytostatic effect of adenine-arabinoside (Ara-A) and cytosine-arabinoside (Ara-C)[1]

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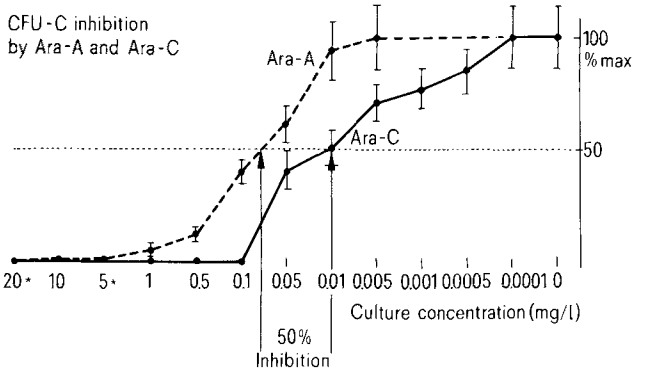
Summary. Adenine-arabinoside, a new antiviral drug with questionable bone marrow toxicity, inhibits colony formation by myeloid precursor cells in vitro. Compared to cytosine-arabinoside this cytotoxicity is roughly one third.

Adenine-arabinoside (Ara-A) is a new and promising antiviral agent that is particularly active against DNA-viruses of the Herpes group[3-10]. It is generally assumed that its antiviral activity greatly outweighs its myelotoxicity in contrast to other antiviral drugs[3]. Marrow depression by Ara-A, however, has been described[11]. The myelotoxicity of Ara-A is difficult to evaluate in clinical situations. Wide use of the drug is hampered by teratogenic side effects, reported in rodents[12]. Its application in man is therefore restricted to life-threatening Herpes virus infections. As these occur in diseases which themselves are likely to interfere with bone marrow function, possible myelotoxic side effects of Ara-A are difficult to document objectively. We therefore chose an in vitro technique to test the myelotoxicity of Ara-A.

Human myeloid precursor cells (CFU-C = colony forming units in culture) form granulocyte and macrophage colonies in culture. This in vitro growth is extremely sensitive to cytostatic agents and is thus an appropriate target for the investigation of drugs with questionable cytotoxicity, such as Ara-A. Cytosine-arabinoside (Ara-C), a chemically related compound of known cytotoxicity, served as reference substance.

10^5 nucleated human bone marrow cells from 10 normals and 6 patients with non-haematological diseases were cultured in methylcellulose as described by Iscove[13]. Ara-A or Ara-C were added in increasing amounts, the highest final culture concentrations corresponding to therapeutic doses. Benzyl alcohol, the solvent used for clinical application of Ara-A was avoided, and culture medium was used instead. After 14 days in culture, granulocyte/macrophage colonies containing more than 20 cells were scored in an inverted microscope.

Mean colony counts of 44 colonies per 10^5 cells were decreased by both Ara-A and Ara-C. The dose-related effect on colony growth is depicted in the figure. It was totally abolished by therapeutic concentrations (indicated by asterisks) of both drugs, 50% inhibition occurred at a concentration which was 8 times higher for Ara-A than for Ara-C. Therapeutic doses, however, are 3 times higher for Ara-A, its actual cytotoxicity is thus roughly one-third compared to Ara-C. We conclude that the cytostatic effect of Ara-A has to be taken into account, particularly if the drug is administered to patients with depressed marrow function.



Dose related inhibition of colony formation by myeloid precursor cells by adenine-arabinoside (Ara-A) and cytosine-arabinoside (Ara-C). Mean values±SEM of 16 experiments on hematologically normal human bone marrow.