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GENE EXPRESSION IN B-CELL LYMPHOMA OF SIV INFECTED Rhesus Monkeys
(M. mulatta). AN OVERVIEW

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Abstract

B-cell Non-Hodgkin’s lymphoma (B-NHL) is a typical tumour after infection with viruses causing immunodeficiency. Both, Simian as well as Human Immundeficiency Virus (SIV and HIV) are well known pathogens suspected to contribute to the development of B-cell lymphoma. Fortunately, macaques infected with SIV were shown to be an adequate non-human primate model for NHL. In our group we established profound pathological examination protocols and identified classification markers. We conducted experiments to further insight into the molecular processes of lymphomagenesis. Presence of simian Epstein-Barr virus and cytokine gene expression patterns in Bcell lymphomas from individual rhesus monkeys (M. mulatta) and from two lymphomas of man were highly similar but not identical and helped to classify these lymphomas on a molecular level. To identify unknown genes differentially expressed in B-cell lymphomas we applied techniques such as „subtractive hybridisation” and in the near future proteome methodology. In conclusion, all data obtained so far recommend the SIV rhesus monkey model for basic research and it may serve as a valid animal model to evaluate therapeutic measures against the human tumour disease.

Zusammenfassung


Résumé

Des lymphomes B cellules Non-Hodgkins (B-NHL) sont des tumeurs typiques qui se produisent après l’infection avec des virus de la déficience immunitaire. Les virus simiens ainsi que humains de la déficience immunitaire (SIV et HIV) sont des pathogènes connus, on craint qu’ils contribuent au développement des lymphomes Bcellules. Au passé on a montré que les singes rhesus infectés par SIV sont un modèle de primates nonhumains fiable. Notre groupe d’études a établi des fiches de contrôle détaillées pour la pathologie et a identifié des marqueurs de classification. Nous avons fait des expériences lesquelles ont enrichi nos connaissances sur les processus moléculaires d’une genèse de lymphome. La présence des virus simiens d’Epstein-Barr et les types d’expression des différents gènes de cytokine étaient à un haut degré comparables dans les B-NHL des singes rhesus et de l’homme, bien qu’ils ne soient pas identiques. Ces expériences servissaient à la classification d’une base biologique moléculaire. Pour dépister des gènes inconnus, qui sont exprimés „differemment” en B-NHL, nous avons utilisé avec succès des dits méthodes de substraction. Désormais nous désirons compléter cette approche expérimentale avec une analyse de protéome. En résumé nous constatons que le modèle B-NHL de singes rhesus ne serve seulement à la recherche fondamentale, mais devrait être également un modèle pour expérimenter sur des futurs stratégies thérapeutiques.
**Key words:** B-cell lymphoma, SIV and HIV, oncogenic herpesvirus, differential gene expression

**Introduction**

Macaques infected with simian immunodeficiency virus (SIV) are thought to be an appropriate model for AIDS – associated human immunodeficiency virus type 1 (HIV-1) infection of humans. These animals also have been found to develop B-cell Non-Hodgkin’s lymphoma (B-NHL) during infection with SIV. Patients with an HIV-1 infection are at high risk to eventually suffer from a high-grade B-NHL. This lymphoma is the most prominent malignancy in HIV infected humans. About 10% of SIV infected macaques proceed to B-NHL (9). Molecular, biological and immunological data indicate that this virus dependent lymphomagenesis is similar in both hosts. In particular, we have shown that intratumoral cytokine gene expression patterns in SIV and HIV-1 lymphomas are comparable but not identical (6,7). Distinct clinical and histomorphological characteristics of human lymphomas were well known and we have extended these studies to the SIV associated lymphomas during the past years.

The SIV-macaque model is unique to study virus dependent tumorogenesis. A number of well known cellular genes playing a role in growth regulation, cell senescence, proliferation and cytokines have been found in the context of lymphomagenesis. However, any causative relationship between these differentially expressed genes and stages of lymphomagenesis is difficult to establish. Consecutive analyses of gene transcription in various cells prone to become a tumour cell would help to define single molecular mechanisms responsible for malignant transformation. In order to identify essential differentially expressed genes in lymphomas we applied immunohistochemistry and investigated transcription of genes suspected to play a role in lymphomagenesis (8). Subtractive hybridisation allowed identifying some genes that were overexpressed in the lymphoma cells (2,11,12,13).

However, a causative role of any deregulated gene needs to be confirmed by highly specific experimental approaches. One should remember that it took some 20 years to be convinced that Epstein-Barr virus is the etiological agent responsible for oncogenic transformation of human B cells.

**Methods to study lymphomagenesis**

Techniques available to detect transcripts of „lymphoma specific genes“ and to identify lymphoma specific genes are key to understand molecular mechanisms. Thus, studies on gene expression rely either on the detection of proteins or on the detection of mRNA transcribed from genes. Quality of isolated mRNA is crucial for detection of transcripts. Many times the difference between basal or background transcription and transcription after induction of gene expression is not as high as one needs for an accurate determination. As long as only qualitative aspects are concerned one succeeds with routine RNA preparations and RT-PCR. These days quantitative assays can be performed as real time assays. This requires efficient isolation with high purity and quality of RNA to be reverse transcribed and its cDNA being amplified. We used for identification of differentially expressed genes subtractive hybridisation techniques. These protocols are not yet ready for routine experiments although companies provide kits for these analyses of differential gene expression. Investigating transcripts and transcript levels is clearly different from detection of protein by immunohistochemistry. Proteins are more stable and persist for a longer time in cells and tissues whereas mRNA is rapidly induced but also may be degraded within short times. Having both techniques at hand is the most promising experimental approach. Today, when proteomics appears to be the most powerful technique to define the phenotype of cells and tissue, one should not forget to establish genetic profiles telling us more about temporal overexpression of genes. Current developments leading to chip formats will allow rapid analyses and patterns of expressed genes will be used to monitor physiological changes taking place inside single cells or tissue. Isolation of RNA from affected tissue and control tissue(s)
is performed with standard techniques. Some protocols are well advanced and allow isolation of sufficient amounts of RNA from small biopsy samples, down to some 10000 cells or less.

**Intratumoral cytokine gene transcription patterns in B-NHL**

In a first set of experiments we performed transcript determination of selected cytokine genes suspected to be major players in the local intratumoral immunosurveillance. Most importantly, transforming growth factor-β (TGF-β) and interleukine 10 (IL-10) mRNA was easily detectable and present in all tumours analysed. This finding supported immunohistochemistry where infiltrating immunocompetent T-cells had been found. Responsiveness of these immunity mediating cells obviously was ablated by these immunomodulators, TGF-β and IL-10. To further this study we also looked for the TGF-β type 1 and 2 receptors. Transcription of the receptor genes and the corresponding proteins had been detected in lymphoma. This experimental approach made us confident that TGF-β does control immune cells since the TGF-β receptors were also present. Unfortunately, there are many studies lacking receptor analysis. Even more, in a cell line originally taken in culture from lymphoma biopsy specimens, we found TGF-β and its receptors. This cell line allowed experiments to look for a function. In doing so, TGF-β appeared to be a negative factor leading to reduced viability and apoptosis. We do not know whether this finding with a cell line can be used to explain intratumoral effects of TGF-β since TGF-β is a pleiotropic factor. Transcripts of the cellular IL-10 were present in all but one tumour. Of note, the latter tumour contained transcripts of a simian Epstein-Barr Virus IL-10 homologue (*Cercopithecine herpesvirus type 15; BCRF 1 gene*). Transcription patterns including interleukine – 2, -4 and -6 as well as TNF –alpha discriminated between centroblastic and immunoblastic B-NHL and one Burkitt’s like lymphoma. All B-NHL were classified according to the Kiel protocol and the REAL classification scheme (3,6,7,9).

**Differential gene expression in B-NHL**

Initial studies aimed at a determination of selected genes with suspected functions controlling intratumoral immunity and malignant growth. To extend this experimental approach we applied techniques appropriate to detect deregulated expression of genes in lymphomas. In principle, RNA from lymphoma tissue and circulating B-cells from a naive animal was isolated and transcribed into complementary DNA (cDNA). After hybridisation only cDNA sequences, indicative for a lymphoma specific over expression, were left (2, 11, 12,13). Extending our studies on the transcription pattern of a number of cytokine genes and growth regulatory factors we succeeded in establishing genetic profiles from SIV associated B-NHL. Several genes have been found to be overexpressed i.e. exhibiting strong transcription in the lymphoma cells.

Some genes belong to growth and differentiation factors that may be suspected to be involved in the process of malignant transformation. One gene, designated SET, is supposed to essentially contribute to this process. Interestingly, abundant transcription of SET gene sequences were also observed in a human HIV associated B-NHL. Genes from the S-100 family could be detected. Members of this group regulate cellular physiology e.g. extracellular signal transduction, adhesion and cancer metastasis. High transcript levels of several mitochondrial genes had been identified, too. None of these genes had been discussed earlier as a cofactor in the lymphomagenesis. All genes together are called oxidative phosphorylation genes (OXPHOS). Perhaps they play a major role in the energy metabolism of cells. Besides, they may profoundly influence apoptosis and tumorogenesis since they seem to interfere with cyclines and p53 controlling cell cycle and growth. In conclusion, investigating transcription programs in B-NHL led to several genes not known and never suspected to be related to lymphomagenesis. Future experiments aim at a description of the phenotype of malignant cells with proteome analyses. We expect this approach to complete our first data on genetic, more precisely transcription profiles.
Persisting herpesviruses as cofactors in B-NHL

The role of persisting viruses in B-NHL has not yet unambiguously defined. Up to 90% of the SIV associated lymphomas of rhesus monkeys harboured simian Epstein–Barr virus (EBV) sequences (*Cercopitheccine herpesvirus 15*). This finding was in line with reports on SIV associated lymphomas of cynomolgus monkeys. As to HIV associated B-NHL, there are only 30-40% EBV DNA positive lymphomas. Presence and expression of simian EB-like viruses most likely shortens survival time of animals; this resembles human EBV infection under viral (HIV-1) induced immunodeficiency. To define any role of the persisting viral genome, we asked whether genes of the simian herpesvirus present in the rhesus monkey lymphomas were transcribed. Two RNA’s, designated EBER, could be detected by in-situ hybridisation in 6 out of 7 lymphomas. The nuclear antigen EBNA 2 was found by immunohistochemistry in 4 out of 7 lymphomas. Interestingly, in one rhesus monkey lymphoma we had barely detectabably levels of cellular IL-10 mRNA. However, this lymphoma contained abundant transcripts of the viral IL-10. It is encoded in the BCRF-1 gene of the human EBV and is also conserved in the simian EBV. As mentioned, the viral IL-10 may compensate the low abundance of cellular IL-10 transcripts. Further studies revealed that another herpesviral gene product, the EBV Latent Membrane Protein 1 (LMP 1) known to directly contribute to malignant transformation, is present in the lymphomas. This result supported our assumption that this gene may definitely influence mechanisms leading to an immortalised stage of cells.

Even more, in 4 out of 9 rhesus B-NHL the full spectrum of latent EBV genes were expressed (EBER ½, EBNA 1 and –2, LMP-1) and thus classified as latency type III. This herpesvirus gene expression pattern also corresponded to that observed in 2 HIV-1 associated B-NHL we had as a control (1, 10). This data strengthened our view that the macaques B-NHL serve as a valuable model for the human B-NHL developing under a virus induced immunosuppression. In addition, we found human herpesvirus type 8 (HHV-8) DNA in a large cell B-NHL in a HIV positive patient (4). Whether HHV-8 had been expressed, could not be analysed. Taken together, the experimental value of the rhesus model is obvious since the time point of SIV infection is known and the status, whether or not the animals carry persistent herpesvirus as a putative cofactor, can be determined before the experiment is initiated.

Results on the soluble CD23 (sCD 23) in serum of SIV infected rhesus monkeys lent support to data on the persistence and expression of herpesviral genes in rhesus B-NHL. The sCD 23 and CD 21 genes were found to be transcribed. The EBNA 2 is known to induce CD 23 and CD 21. Both genes may help to bring about infection of cells with EB type viruses. The CD 21 may be the receptor for EBV and the CD 23 and CD 21 interaction could be pivotal for activation and proliferation of B- cells (5).

Perspectives for basic research, diagnostics and prognosis

Proteomics, a collection of old techniques completed by new laser and computer directed analyses may be a way to further knowledge on the phenotype of malignant cells. Any stepwise process of malignisation can be shown to a certain degree on the level of transcription. Rather rapid changes in gene expression take place when promoters are activated and mRNA is formed. In contrast, sustaining ablation of gene expression over longer periods of times, e.g. many hours, might go undetectable in transcription analyses and need other experimental approaches. Isoelectric focussing where proteins are separated by their charge in a pH gradient and subsequent separation according to size in polyacrylamide gels are the methods of choice in proteomics. Subtle differences in the population of proteins in cells or selected tissues can be identified.

To date, most promising work with these methods has been performed especially in cancer and infection biology. Proteomics for determination of the phenotype and transcriptomics for determination of the genotype of cells and tissue became valuable tools to study differential gene expression. They aid to differentiate between physiologically normal cells and those cells undergoing changes induced by cellular deregulation, viral infection or injured by other causes. Development of these techniques might widen prognostic aspects in the future. Basic clinical examination will be the first step but molecular expression profiles determined on chips might help
to better differentiate pathological changes caused by inflammatory processes, lymphoproliferative events or other disease associated mechanisms.

The non human primate model for B-NHL developing under viral immunodeficiency

In the past our laboratory conducted a number of studies that eventually confirmed the SIV infected rhesus monkey with B-NHL as a reliable model for the human HIV-1 associated B-NHL. Besides diagnostics of SIV infected animals all these clinical and molecular approaches may be of relevance for human disease. Experimentally, a defined dose of virus given at a certain time point allows determination of early events leading to malignancy. Usually, human B-NHL is detected but the time point when virus had infected the organism remains unknown. Thus, clinical and molecular data are taken from late stages of infection and the B-NHL. In contrast, well-defined infections with pathogens, presence and gene expression of pathogens, therapeutic measures and other intervention strategies can be investigated in many more details in such a non-human primate model.

References

6. Elevated levels of sCD23 in sera of SIV-infected rhesus monkeys developing Non-Hodgkin's lymphoma.

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MALIGNANT LYMPHOMAS IN SIV INFECTED RHESUS MONKEYS (*Macaca mulatta*)

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**Abstract**

Malignant lymphomas are naturally occurring neoplasms in rhesus macaques (*Macaca mulatta*) and in other nonhuman monkeys. Usually their incidence is very low, but deficiency and dysregulation of the immune system seem to increase their frequency. According to that, malignant lymphomas are frequently observed in the late stage of simian immunodeficiency virus (SIV) infected rhesus monkeys. This report summarises the three major types of SIV-associated lymphomas in infected rhesus macaques and demonstrates the clinical manifestation, the histopathology and the close correlation to EBV-like simian herpesvirus (sEBV/Lymphocryptovirus).

**Zusammenfassung**


**Résumé**

Les lymphomes malins sont des néoplasmes survenants naturellement auprès des macaques rhesus (*Macaca mulatta*) et d’autres espèces de singes nonhumains. D’habitude leur incidence est peu important, mais il semble que la déficience et la dysrégulation du système immunitaire augmentent leur fréquence. En conséquence on observe des lymphomes malins souvent au dernier stade des singes rhesus infectés par le virus d’immunodéficience simien (VIS). Ce rapport résume les trois types majeurs de lymphomes associés à VIS auprès des singes rhesus infectés et démontre la manifestation clinique, l’histopathologie et la correlation étroite au virus d’hépatite simien semblable à EBV (sEBV/Lymphocryptovirus).

**Key Words:** malignant lymphoma, rhesus macaque, simian immunodeficiency virus (SIV), Kiel classification, simian equivalent of Epstein-Barr virus (sEBV)

**Introduction**

The frequency of malignant lymphomas in non human primates seems to increase with deficiency and dysfunction of the immune system as it is observed in simian immunodeficiency virus (SIV) infected rhesus macaques (6,7,11,20). Histopathological examination revealed that the lymphoid neoplasms are mainly of B-cell origin and develop similar to the malignant lymphomas in human immunodeficiency (HIV) infected man (1,12,16). They are characterised by disseminated extranodal growth in different organs with predilection for viscera. The highest number of these B-cell lymphomas belongs to the two types of non-Hodgkin lymphomas known from the Kiel classification (2): i) centroblastic lymphoma of monomorph subtype or centroblastic lymphoma of polymorph subtype and ii) immunoblastic lymphoma with or without plasmacytic differentiation.
Another frequently observed B-cell lymphoma is the Burkitt-like lymphoma. This tumour usually consists of a monomorphous cell population interspersed by multinucleated giant cells and macrophages with starry-sky appearance. Here we present our experience with SIV associated lymphomas in rhesus monkeys.

Clinical course of infection and gross pathology

Affected animals with B-cell lymphoma show similarity in their clinical course. Gradually they develop generalised lymphadenopathy and moderate to severe weight loss. Their CD4/CD8 lymphocyte ratio in blood is markedly decreased and serologically they are positive for simian Epstein-Barr virus (sEBV). Indicative for immunodeficiency or -suppression a number of animals suffer from opportunistic infections mainly caused by *Pneumocystis carinii*, *Cryptosporidium* sp. or *Cytomegalovirus*. At necropsy the lymphomas are generally found in several disseminated extranodal locations. Primary growth is observed in visceral organs, including thymus, liver, spleen, kidney, colon and small intestine, but in some cases even heart, nose, eyelid, or testes are involved. In general, the monkeys also develop massive follicular hyperplasia of various tumour-free lymph nodes and severe splenomegaly.

Histopathological findings

Histopathological examination including immunohistochemical studies show that the observed tumours are high-grade malignant B-cell lymphomas. Using the Kiel classification it is possible to divide the non-Hodgkin B-cell lymphomas into two different groups, each with its predominant characteristic tumour cell.

1) * Centroblastic lymphoma of monomorph subtype:*
   The main cell type is represented by monomorph round centroblasts (CD20 positive).
2) * Centroblastic lymphoma of polymorph subtype:*
   Centroblasts and 10% of immunoblasts determine the histological picture (CD20 positive).
3) * Immunoblastic lymphoma with or without plasmacytic differentiation:*
   Typical tumour cells are immunoblasts (CD20 positive) varying in degree of plasmacytic differentiation.

The Burkitt-like lymphoma is characterised by a monomorphous B-cell population (CD20 positive) infiltrated by macrophages containing cellular debris and giving the "starry sky" appearance (CD68 positive). Tumour-infiltrating multinuclear giant cells, positive for CD68, have been observed in some cases. The expression of the proliferation marker Ki67 is up to 90% positive in all of these tumours.

Discussion

Malignant lymphomas are usually less observed in nonhuman primates, but in association with SIV as immunosuppressive agent they have been seen more frequently (1,6,19). The tumour incidence in different SIV-infection studies ranges from 6 to 83% depending on the viral strains employed for the experimental infection and the individual status of the monkeys (species, age, sex etc.) (1,7,9,13).

This report focuses on malignant lymphomas with the highest frequency. As they are mostly classified as high-grade malignant B-cell lymphomas, they are more obviously differentiated by the Kiel classification. The Kiel classification as widely used scheme to distinguish the different human malignant lymphomas divides the non-Hodgkin lymphomas (NHLs) of B-cell phenotype into two main groups:

1) centroblastic lymphoma of monomorph subtype and centroblastic lymphoma of polymorph subtype; 2) immunoblastic lymphoma with or without plasmacytic differentiation.

The Burkitt-like lymphoma is characterised by a compact monotonous cell population with infiltrates of single histiocytes of "starry sky" appearance.
When the REAL (Revised European American Lymphoma) classification is applied, the observed neoplasms’ are not subdivided into the proposed subtypes and are classified as diffuse large B-cell lymphomas (8,10).

Predominant locations of the lymphomas have not been reported. In general, they are distributed in two or more extranodal tissues accompanied by massive follicular hyperplasia of tumour-free lymph nodes and by splenomegaly (7,16).

With respect to the current opinion on the development of the SIV-induced malignant lymphoma, it is probably the result of a sustained B-cell proliferation caused by a continuous T-cell decline and various participating cytokines (4,17,21). Opportunistic infections may enhance and accelerate these processes.

As in HIV-infected humans, CMV, EBV and HHV-8 are suspected to be additional pathogenic factors in lymphomagenesis, CMV and sEBV (simian equivalent of Epstein-Barr virus) seem to play a similar role in SIV-infected monkeys (5,6). Searching for these co-factors, various observed animals had CMV and sEBV antigens in tumours and tumour-neighbouring tissues and may support this hypothesis (3,16,18). The major role of EBV most likely results from a defect in the growth regulation of EBV-transformed lymphocytes (5). Similar to EBV infections of man another EBV-like herpesvirus associated with malignant lymphomas in SIV-infected cynomolgous macaques has been found and assigned as herpesvirus HVMF 1 (herpesvirus *Macaca fascicularis*) (14,15). The essential role of the sEBV and any EBV-related herpesvirus is probably due to their lymphotropic character and their oncogenic potential, inducing massive proliferation and outgrowth of B-lymphocytes (16). Further studies are needed to establish the responsible viral genes, that control the malignant cell transformation in immunocompromised animals.

**References**


MALIGNANT LYMPHOMA IN TWO VICUÑAS (Lama vicugna)

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Abstract

A 13-year-old and a 10-year-old female vicuña (Lama vicugna) in the Zurich zoo died after a history of non-specific clinical symptoms including diarrhoea, emaciation, and being chased away by the breeding male. At necropsy, nodules of variable diameter were found in lung, myocardium, jejunum, liver, and kidneys of animal 1, and in lung, mediastinum, pericardium, and pleura of animal 2. Based on the histomorphological and immunohistochemical features, a malignant lymphoma of T-cell origin was diagnosed in animal 2 and of questionable T-cell origin in animal 1. The two affected vicuñas originate from the same parent animals and a genetic cause of the disease has to be considered.

Zusammenfassung


Résumé

Une femelle vigogne (Lama vicunia) de 11 ans et une de 10 ans sont mortes au zoo de Zurich après avoir montré des symptômes non spécifiques tels que diarrhée, émaciation et rejet par le mâle reproducteur. L’autopsie a dévoilé des nodules de diamètre variable dans le poumon, le myocarde, le jéjunum, le foie et les reins de l’animal 1 et les poumons, le médiastin, le péricarde et la plèvre de l’animal 2. À partir des caractéristiques histomorphologiques et immunohistochimiques des prélèvements, un lymphome malin ayant pour origine les lymphocytes T a été diagnostiqué chez l’animal 1, mais la même origine chez l’animal 2 restait douteuse. Les deux vigognes touchées descendent des mêmes parents et une origine génétique doit donc être envisagée.

Key words: Vicuña, Lama vicugna, lymphoma, lymphosarcoma, neoplasia

Extended abstract

Case report

Animal 1, a 13-year-old female vicuña (Lama vicugna) born at the Zurich Zoo, had a previous history of irregular birth intervals as well as stillbirths. Within the last months prior to death there were several observations that the animal was chased off by the breeding male. In January 1999,
the animal showed emaciation and profuse watery diarrhoea. Coprological culture and parasitology were negative for pathogenic organisms and the condition was resolved with symptomatic treatment. Four days later the same animal displayed severe lameness of its left hindleg and clinical examination revealed a septic tarsitis. Arthrocentesis exhibited elevated synovial leukocyte counts as well as cultural growth of Clostridium septicum. An arthrotomy was performed under general anaesthesia and the joint was cleaned of fibrinous debris and flushed with lactated Ringer’s solution and antibiotics. The animal was found dead one day after surgery.

Animal 2, a 10-year-old female vicuña (Lama vicugna) also born in the Zurich zoo, had a history of being chased off by the breeding male for several months. Bite wounds in the neck, emaciation and diarrhoea were noted on several occasions and ascribed to this social interaction. Regular coprological investigations were inconclusive with the exception of cultural growth of *Salmonella enterica* subsp. *enterica* in one sample. The animal died unexpectedly in July 2001.

In gross pathology, both animals showed emaciation, moderate to severe serous pleural and peritoneal effusions as well as multiple pale white, firm nodules of variable diameter with occasional central softening scattered throughout all lung lobes. Thoracic lymphnodes were enlarged in both cases. Additionally, animal 1 showed a single pale white firm nodule (Ø 10 mm) in the myocardium of the left atrium and multiple pale white, nodular, firm masses (Ø 2 – 15 mm) in liver, jejunum, and kidneys. In animal 2, pathological findings were confined to the thoracic cavity and were characterised by multiple pale white, firm to centrally softened nodules of variable size in mediastinum, pericardium, and pleura costalis.

Histologically, the nodules consisted of dense, invading proliferations of large, round to spindle-shaped cells. The mostly large nuclei were ovoid, cleaved or convoluted and exhibited a clumped chromatin pattern and occasionally visible nucleoli. There was a moderate to high amount of eosinophilic or amphophilic cytoplasm. Apoptotic cells and mitotic figures were numerous in certain areas.

Immunohistochemically, a small percentage of the neoplastic cells showed a questionable weak CD3-reaction (a pan T-cell marker) in animal 1, and the majority of the neoplastic cells was moderately CD3-positive in animal 2. The lysozyme-reaction (a macrophage marker) and the CD79a-reaction (a B-cell marker) were negative for both animals.

Based on the histomorphological and immunohistochemical features, a pleomorphic large-cell lymphoma of T-cell origin was diagnosed in animal 2 and of possible T-cell origin in animal 1.

**Discussion**

Malignant Lymphoma or lymphosarcoma has been described in many species of large animal (5,8,12), and may be the most common malignant neoplasm in many of these species (1,3). Although neoplasms are uncommon in South American camelids (SAC) in general, there are several anecdotal reports of malignant lymphoma in the domestic forms llama and alpaca (6, 7, 10, 11). Recently, the medical records of 1,156 llamas and alpacas at Colorado State University were reviewed and malignant neoplasm’s were histopathologically confirmed in 12 cases (1%), and malignant lymphoma (n=5, 0.4%) represented the most common neoplasm seen in this veterinary hospital (4).

To our knowledge, the present report documents the disease in vicuñas for the first time. Interestingly, the two cases originate from the same parent animals and a familiar disposition is possible. A genetic basis for the sporadic form of malignant lymphoma is discussed in cattle (9). This would implicate serious consequences for breeding programs of captive vicuñas and necropsy reports therefore have to be carefully monitored in these breeding lines. However, the mother of the two animals died in Zurich and no incidence of neoplasia was found at necropsy. The father was transferred to another institution and is still alive.

An antemortem diagnosis was not achieved in these two cases and the clinical signs were non-specific or masked by other conditions. Weight loss and peripheral lymphadenopathy were consistent findings in a retrospective study of llamas and alpacas (4). In both vicuñas only thoracic lymphnodes were found to be enlarged. Lymphadenopathy is a particularly noteworthy finding in SAC because their peripheral lymphnodes are normally small and difficult to detect by palpation; fine needle aspiration would be recommended for antemortem diagnosis. An interesting feature in both animals was the observed chasing off by the breeding male trying to exclude the affected
animals from the herd. This may be interpreted as a first sign of disease and attentive observation should be the rule when evaluating social behaviour in captive vicuña groups. Multifocal nodular masses with varying organ distribution were a consistent finding in gross pathology in these two vicuñas. In conventional ruminant species, cutaneous, multicentric, thymic, and alimentary forms of lymphoma have been described (2,8). These are named after the predilection sites for tumour growth. Histology clearly indicated the aggressive character of the condition and immunohistochemistry showed a questionable CD3-reaction in animal 1 whereas animal 2 was moderately positive for this T-cell marker. Questionable CD3-reactions can be found in T-cells with poor differentiation (T-zero-cells). In summary, this is the first report of malignant lymphoma in the vicuña. Clinical symptoms were non-specific and the diagnosis was based on post-mortem findings. Because the two affected animals originated from the same parent animals, a genetic basis is possible, and increased awareness of zoo personnel responsible for captive vicuña herds and breeding programs is imperative.

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References

DIAGNOSIS AND SURGICAL TREATMENT OF HYPERTHYROIDISM DUE TO THYROID ADENOMA IN A GREEN IGUANA (Iguana iguana)

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Abstract

Confirmed cases of thyroid neoplasia (adenomas and carcinomas) are rare in reptiles. A desert tortoise (Gopherus agassizii) died from multiple pathologic and metabolic processes, including parathyroid adenomas and papillary thyroid colloid adenoma (1). Thyroid adenocarcinoma was diagnosed at necropsy in a crocodile lizard (Shinisaurus crocodilurus) that presented with anorexia and lethargy (2). Thyroid adenomas or carcinomas have also been reported in an African sungazer lizard (Cordylus polyzonus), Komodo dragon (Varanus komodoensis), painted turtle (Chrysemys picta) and water monitor (Varanus salvator) (3, 4, 5). Hyperthyroidism has been suspected in snakes that undergo very frequent ecdysis. These reports have been largely anecdotal and lacked TRH, TSH, or T₄ evaluation (6). There have been no previous reports of hyperthyroidism in the Sauria or Crocodilia. This report describes a case of hyperthyroidism due to thyroid adenoma in an adult female green iguana (Iguana iguana). The lizard presented with polyphagia, loss of the dorsal spines, hyperactivity, and increased aggression. Physical examination revealed tachycardia and a bilobate mass palpable anterior to the thoracic inlet. Clinical investigation included haematology, plasma biochemistry, radiography, and ultrasonography. Diagnosis of hyperthyroidism was based on a total T₄ (30.0 nmol/L [2.34 µg/dL]) elevated above that of clinically healthy iguanas (3.81 ± 0.84 nmol/L [0.29 ± 0.07 µg/dL], n = 7), and histopathology confirmed a functional thyroid adenoma. Surgical thyroidectomy proved both safe and effective in returning the lizard to an euthyroid state.

Zusammenfassung

Bei Reptilen sind bestätigte Fälle von Schilddrüsenneoplasien selten. Eine Wüstenschildkröte (Gopherus agassizii) starb durch mehrfache pathologische und metabolische Prozesse, einschließlich Adenome der Nebenschilddrüse und Adenome des papillären Schilddrüsen Kolloides. Thyroidadenocarcinom wurde diagnostiziert bei einer Nekropsie einer Krokodilschwanz Höckerechse (Shinisaurus crocodilurus), welche angeboten wurde mit Anorexie und Lethargie (2). Thyroidadenome oder –carcinome wurden auch beschrieben bei einer afrikanischen Sonnenanbeter Gürtelschweif (Cordylus polyzonus), bei Komododrachen (Varanus komodoensis), bei einer Zierschildkröte (Chrysemys picta) und bei einem Bindenwaran (Varanus salvator) (3, 4, 5).

Der Verdacht auf Hyperthyroidie besteht bei Schlangen, die sich frequent häuten. Diese Berichte kommen vereinzelt vor und es fehlt eine TRH, TSH oder T₄ Auswertung (6). Es gibt noch keine Berichte über Hyperthyroidie bei Sauria oder Crocodilia. 

Dieser Bericht beschreibt einen Fall von Hyperthyroidie durch ein Thyroidadenom in einem ausgewachsenen weiblichen grünen Leguan. Die Echse zeigte Polyfagie, Verminderung der dorsalen Dornen, Hyperaktivität und erhöhte Aggressivität. Bei einer physischen Untersuchung wurde Tachycardie und eine bilobate Masse fühlbar vor dem Brusteingang. Die Klinischen Untersuchungen umfassten auch Hematologie, Plasma Biochemie, Radiographie und Ultrasonographie. Die Diagnose Hyperthyroidie wurde basiert auf einen total T₄ Wert (30,0 nmol/L [2,34 µg/dL]), der höher war, als bei gesunden Leguanen (3,81 ± 0,84 nmol/L [0,29 ± 0,07 µg/dL], n=7) und histopathologie, die ein funktionierendes Thyroidadenom bestätigte. Mit Hilfe einer chirurgischen Thyroidectomie wurde die Echse sicher und effectief wieder in eine euthyroidie Situation gebracht.

Résumé

Les cas confirmés de métaplasie thyroïdienne (adénomes et carcinomes) sont rares chez les reptiles. Une tortue Gophere (Gopherus agassizii) est morte de multiples troubles pathologiques et métaboliques, dont un adénome
parathyroïdien et un adénome colloïde thyroïdien papillaire(1). Un adénocarcinome thyroïdien fut diagnostiqué à l’autopsie d’un Shinisaure crocodilure (Shinisaurus crocodilurus) qui présentait des signes d’anorexie et de léthargie (2). Des carcinomes thyroïdiens ont également été rapportés chez le Cordyle africain (Cordylus polyzonus), le Dragon de Komodo (Varanus komodensis), la Chrysemyle peint (Chrysemys picta) et le Varan a deux bandes (Varanus salvator) (3,4,5).

L’hyperparathyroïdie chez le serpent a été mise en cause chez les animaux montrant de fréquents signes d’ecdysie. Ces rapports de cas sont très anecdotiques et dépourvus de mesures de TRH, TSH ou T4 (6). Aucun rapport d’hyperparathyroïdie chez les sauriens ou les crocodiliens n’est connu à ce jour. Ce rapport décrit un cas d’hyperthyroïdie dû à un adénome thyroïdien chez une femelle Iguane Vert (Iguana iguana) adulte. L’iguane montrait des signes de polyphagie, perte d’épines dorsales, hyperactivité, hyperagressivité.

L’examen clinique a révélé une tachycardie et une masse bilobée palpable crâniamente à l’entrée de la cage thoracique. Les examens complémentaires qui ont été entrepris incluent hématologie, biochimie, radiographie, échographie. Le diagnostic d’hyperparathyroïdie repose sur les dosages des T4 total (30 nmol/L, [2.34 mg/dL]), plus élevés que les valeurs mesurées chez les iguanes cliniquement sains (3.81±0.84 nmol/L, [0.29±0.07 mg/dL], n=7) et l’histologie a confirmé un adénome thyroïdien fonctionnel. La thyroïdectomie chirurgicale s’est montrée un moyen efficace de rétablir l’animal.

**Key words:** *Iguana iguana*, thyroid adenoma, hyperthyroidism, thyroidectomy.

**References**

THYROID ADENOMA IN A STINKPOT (Sternotherus odoratus)

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Abstract

Clinical symptoms, diagnostic procedure and results of the necropsy of a stinkpot (Sternotherus odoratus) with a thyroid adenoma are described.

Zusammenfassung

Es wird die klinische Symptomatik, die Diagnosestellung und die Sektionsbefunde einer Moschusschildkröte (Sternotherus odoratus) mit einem Schilddrüsenadenom dargestellt.

Résumé

Symptômes cliniques, méthode de diagnostic et résultats de nécropsie d’un putois (Sternotherus odoratus) atteint d’un adénome thyroidien.

Key words: chelonians, turtles, stinkpot, Sternotherus odoratus, thyroid, neoplasia, adenoma

Introduction

Neoplasms of all the major body systems have been reported in chelonia. Several reviews on neoplasia in reptiles have been done (1, 4, 5, 7, 6). Machotka (1984) has mentioned a thyroid adenoma in a fresh water turtle (Pseudemys geoffranus). One case of a thyroid carcinoma in a Ceylon terrapin (G. trijuga) has been described by Cowan (1968).

History

A 15-year-old female stinkpot (Sternotherus odoratus), weighing 523 g and showing a carapace length of 12 cm, was presented in our clinic in summer 2001, as it had been suffering from apathy, anorexia and oedema all over the body for about 10 days. The turtle was housed in a tank together with 4 other turtles of different species. Blood-work and ultrasonography were performed. Due to the poor general condition and the infaust prognosis the animal was euthanised. Afterwards necropsy including histology was performed.

Results

Haematological examination revealed anaemia (PCV 9%), whereas the other parameters were in reference range.
(WCBC: 4,000 leukocytes/µl, uric acid: 3.2 mg/dl, calcium: 15.97 mg/dl, phosphorus: 5.47 mg/dl, BUN 15 mg/dl, ALT 10 U/l, AST 55 U/l).

Ultrasonography revealed a hypertrophy of the thyroid, characterised by a cystic structure. Necropsy showed a degenerated thyroid with a diameter of 5 cm and an alveolar-like structure. Subcutaneous tissue all over the body was oedematous. Other organs showed no abnormal findings. Histopathology revealed an adenoma of the thyroid with a mild granulocytic infiltration.

Discussion

Long lasting inappetence (famine oedema), nephropathy (3) or cardiac disease (8) may cause oedema in chelonians. So as a differential diagnosis a disease of the thyroid should always be considered, too. In the present case, mechanical obstruction of the heart due to the extreme hypertrophy of the thyroid, may have caused the oedema. Due to empirical experience, diseases of thyroid are not rare in chelonians and should find more attention.

References

PANCREATIC CARCINOMA IN A BROWN BEAR - CASE REPORT

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Abstract

A Brown Bear aged 22 years displayed progressive anorexia, vomiting, jaundice and blood in the feces. Therapy was successful only periodically. Clinically a hepatitis associated with icterus, gastrointestinal ulcer and tumor were assumed. Postmortem examination of the bear revealed a pancreatic carcinoma with obstruction of the bile duct and an ulcer of the duodenum.

Zusammenfassung


Résumé

Un ours brun d’Europe souffrait d’inappétence, de vomissement, des fèces noires et de jaunisse. La thérapie symptomatique n’avait que des succès temporaires. Le diagnostic clinique supposait une hépatite avec icterus, ulcères gastriques dues probablement par un néoplasme. La pathologie revelait un carcinome du pancréas avec une obstruction biliaire et une ulcération du duodénum.

Keywords: tumor, neoplasia, carcinom, pancreas, icterus, bear.

Introduction

The female Brown Bear „Selma“ (Ursos arctos arctos) was born on January 4th 1979 in Kolmarden Animal and Natural Park, Sweden. She became the most famous bear in Switzerland on December 12th 1998. A drunken man, a surgeon, treated her with snowballs. Less snow available and reduced attendance by the bear made him tired and he slipped down on the inner side of the fence he climbed over in the beginning. „Selma“ realizing the aggressor in reach, attacked and pulled him down to the floor of the Bernese Bear Pit. After making clear to „Urs“, the male Brown bear in the pit, that this object was her meal she dragged the man down to her „cave“. The keeper, realizing the danger called for police run down to the caves and made some ugly noise with a metal tube slamming against the iron bars of the cave. „Selma“ being irritated by her beloved keeper left her „dish“ and went out of the cave, the man was saved. Neither „Selma“ nor Berne Animal Park have ever heard from him since that time.

Brown Bears have been kept in Bernese Bear Pits since the middle ages. The present bear pit was built in 1857 and reconstructed in 1994-96. The bears are fed with seasonal fruits, greens and...
vegetables, branches, honey, nuts, fish, eggs, chicken and mineral bear cubes. Protein contents were reduced heavily over the past years. The bear cubes contain calcium, phosphor, vitamins A, D and E among others. As a matter of behaviour enrichment the feed is being hidden over the whole bear pit. The bears are sleeping within their caves on straw-nests.

Case Report

During winter 2000/2001 „Selma“ showed lack of appetite, not very uncommon in winter times. During May 2001 she ate less and started regurgitating. For two days the bear slept day and night. Afterwords she fed normally and feces became normal too. In the end of may the bear showed similar symptoms as before and therefore was treated with corticosteroids and antibiotics (penicillin-streptomycin) for three days. On June 3rd „Selma“ was laying in the bear-pit apathically and the keeper didn’t manage to bait her into the stall. The bear was treated again with the same drugs using blow-pipe. After one day she walked into the stall, staggering. On day three after, she regurgitated coagulated blood and was treated with antibiotics for another week. The bear started feeding again but the feces became black and greasy. Vitamine ADE and B-complex were applied without significant result.

On June 20th „Selma“ was anesthesized using 2 ml Ethorphin (Immobilon®) and 100 mg Ketamine (Ketasol®). After 45 minutes an additional dose of 0.5 ml Immobilon® was given. After 80 minutes the bear received 3.0 ml of Revivon® and got on his feet after 7 minutes. After 5 hours he received an additional dose of 3 ml Revivon® i.m. Results of the examination: Jaundice displayed on all mucous membranes. Palpation of the abdomen revealed an intraabdominal effusion. A blood sample was taken from the sublingual vene and the cavum abdominale was punctured. Results of the blood examination were: leucocytosis, hypoproteinemia, hyperbilirubinemia, increased kidney and liver values, esp. GGT and AP (tab. 1 and 2).

<table>
<thead>
<tr>
<th>Table 1: Hematological results</th>
</tr>
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<tbody>
<tr>
<td>Hct (l/l)</td>
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<tr>
<td>Hgb (mmol/l)</td>
</tr>
<tr>
<td>RBC (10e12/l)</td>
</tr>
<tr>
<td>MCH (fmol)</td>
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<td>MCV (fl)</td>
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<td>MCHC (mmol/l)</td>
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<td>WBC (10e9/l)</td>
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<td>Normo (%)</td>
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<tr>
<td>Neut.band. (%)</td>
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<td>Neut.seg. ()&amp;</td>
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<td>Eos (%)</td>
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<td>Mono (%)</td>
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<td>Lymph (%)</td>
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<tr>
<td>Normo (10e9/l)</td>
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<td>Neut.band. (10e9/l)</td>
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<tr>
<td>Eos (10e9/l)</td>
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<tr>
<td>Mono (10e9/l)</td>
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<tr>
<td>Lymph (10e9/l)</td>
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<td>Platelets (10e9/l)</td>
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### Table 2: Serum biochemistry

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<tr>
<td>Na (mmol/l)</td>
<td>135</td>
<td>139</td>
</tr>
<tr>
<td>K (mmol/l)</td>
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<td>4.7</td>
</tr>
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<td>Ca (mmol/l)</td>
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</tr>
<tr>
<td>Cl (mmol/l)</td>
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<td>104</td>
</tr>
<tr>
<td>P (mmol/l)</td>
<td>1.83</td>
<td>1.55</td>
</tr>
<tr>
<td>Fe (umol/l)</td>
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<td>30</td>
</tr>
<tr>
<td>Gluc (mmol/l)</td>
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</tr>
<tr>
<td>Cholest. (mmol/l)</td>
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<td>6.71</td>
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<td>Total Prot. (g/l)</td>
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<tr>
<td>Albumin (g/l)</td>
<td>26.3</td>
<td>44.8</td>
</tr>
<tr>
<td>BUN (mmol/l)</td>
<td>8.61</td>
<td>5.5</td>
</tr>
<tr>
<td>Creatinin (umol/l)</td>
<td>168</td>
<td>247</td>
</tr>
<tr>
<td>Bilirubin (umol/l)</td>
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<td>1.4</td>
</tr>
<tr>
<td>ALAT/GPT (IU)</td>
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</tr>
<tr>
<td>ASAT/GOT (IU)</td>
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<tr>
<td>AP (IU)</td>
<td>617</td>
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<tr>
<td>CK (IU)</td>
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</tr>
<tr>
<td>g-GT (IU)</td>
<td>746</td>
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<tr>
<td>GLDH (IU)</td>
<td>50</td>
<td>4</td>
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<tr>
<td>LDH (IU)</td>
<td></td>
<td>499</td>
</tr>
</tbody>
</table>

Titer for leptospirosis was negative. Examination of the feces: neither salmonella nor campylobacter or chlostridium were found. The yellow punctuat of the abdomen was posive for erythrocytes and some neutrophile granulocytes. 
Clinical diagnosis was hepatitis, icterus and ulcus of the stomach based on a neoplasia, presumably. Therapy during narcosis consisted of Ringer-Lactat i.v., Penicillin-Streptomycin s.c. and Sucralfat (Ulcogant®, Merck) p.o.
After therapy (antibiotics, Ulcogant® in sweet milk) „Selma” developed well, fed good and routinely. In addition feces were normally. From june 26th 2001 on the bear displayed again anorexia, ataxia and black and greasy feces. Her condition became worse and therfore „Selma” was euthanized on june 28th.

**Pathology**

The bear was in good condition and weighed 115 kg. The mucosae and the fat tissue were bright yellow, indicating severe icterus.

The liver had an increased consistency, the external surface was smooth and the cut surface showed a yellowish marbled pattern. The gall bladder and the ducti cysticus, hepaticus and choledochus were massively dilated, had a thickened wall and were filled with bile (about 2 liters) (Fig. 1).

Within the duodenal wall there was a firm, lobulated, circumscribed nodule arising from the pancreas and which was surrounding and compressing the opening of the bile duct. This nodule was 6 centimeters in diameter, was whitish in color and had a cut surface with scattered hemorrhagic necrotic areas. The surface in the duodenum lumen was diffusely ulcerated and hemorrhagic and the papilla duodeni was swollen and protruding into the lumen.
Histologically, the nodule was subdivided into large lobules separated by thick trabecules of fibrous tissue. The lobules were composed of nests and cords of epithelial neoplastic cells arranged in acinar and tubular structures intermingled by delicate fibrovascular stroma. The neoplastic cells were fairly good differentiated, with finely granular, eosinophic cytoplasm in the apical region and a basal, oval, vesicular nucleus of varying size. Mitotic figures were observed frequently (2 to 5 per HPF). The tumor was not encapsulated and was invading the duodenal wall and the normal pancreatic tissue. No metastases were observed in the surrounding tissues and lymphnodes.

The lesions in the liver were characterized by a moderate biliary fibrosis associated with a prominent bile duct proliferation and a mononuclear cell infiltrate. There was a marked canalicular cholestasis with distention of the canaliculi and formation of bile plugs. Granular brown bile pigment was also present within the hepatocytes. Multifocally necrosis of groups of hepatocytes and mild infiltration with neutrophils were recorded.

Other necropsy findings included a mild multifocal purulent pyelonephritis and degenerative joint disease with thinning of the articular cartilage and periarticular osteophyte proliferation in several joints, especially in the coxo-femoral joints, knees and tarsal joints.

**Discussion**

Maligne neoplasia in large bears are often described in literature. Survey is given by Hellman et al. (3). Three cases of pancreatic carcinoma are available: one case in a Codiac-Bear (*Ursos arctos middendorffi*) (5), one case in a bear of unknown species (6) and on case in a Collared Bear (*Ursus thibetanus*) (2). One Polar Bear (*Thalarcos maritimus*) had adenomas and carcinomas of the pancreatic islets(1).
Pancreatic carcinoma are supposed to be caused by carcinogenic substances too. Experimentally this thesis was proven for nitrosamines to be a azine pancreatic carcinogen in the Golden Hamster. Lack of selenium increases the susceptibility. Beverages containing methylxanthine are discussed to increase the risk of pancreatic carcinoma in man (4). For the carcinogenesis oxidative stress is supposed to be important. Vitamines A, C and E influence on the metabolism of the oxygen radicals and the growth of the tumors. This is proven for the Golden Hamster (7).

There is no record of tumors in the Bernese Bears even though they became quite old. Examination for the carcinogenetic substances mentioned has never been performed. Maintainance with vitamines seems to be suffienct because of the fruits, greens, nuts and dietary supplements for the bears.

In our case the tumor obstructing the bile duct was fatal. Surgery was impossible because of the circular position of the neoplasia. Because of the regurgitation oral therapy was jeopardized. Daily application of drugs using blow-pipe bame more and more a problem of animal welfare as like the pain and anorexia. Therefore euthanasia became imperative.

References

AN OLFACTORY NEUROBLASTOMA (ESTHESIONEUROEPITHELIOMA) IN A BLUE-TONGUED SKINK (Tiliqua scincoides) WITH INVOLVEMENT OF THE JACOBSON'S ORGAN. A CASE REPORT

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Abstract

A blue-tongued skink (Tiliqua scincoides) suffered from a tumour, which occupied the right nasal cavity up to the right eye and protruded through the palatum into the oral cavity. The tumour could not be removed and the animal was euthanised. A well-differentiated esthesioneuroepithelioma of the olfactory epithelium with involvement of the Jacobson's organ was diagnosed at histology.

Zusammenfassung

Bei einem Blauzungen-Skink (Tiliqua scincoides) wurde ein Tumor festgestellt, den rechten Nasengang bis zur Augenhöhle ausfüllte und durch den Gaumen bis in die Mundhöhle gewuchert war. Der Tumor konnte nicht operativ entfernt werden. Das Tier wurde eingeschläfert. Die histologische Untersuchung ergab das Vorliegen eines gut differenzierten Ästhesioneuroepithelioma's, in das das Jacobsonsche Organ mit einbezogen war.

Résumé

Un scinque géant (Tiliqua scincoides) était atteint d'une tumeur qui remplissait la narine droite jusqu'à l'orbite et proliférerait jusque dans la cavité buccale, traversant le palais. Il n'était pas possible d'exciser la tumeur et l'animal a été euthanasié. L'examen histologique a révélé la présence d'un esthésioneuroépithéliome bien différencié impliquant l'organe de Jacobson.

Key words: Blue-tongued skink, Tiliqua scincoides, tumour, neoplasia, nose

Introduction

Olfactory neuroblastomas are uncommon tumours, originating from the neuroectoderm in the nasal cavity (3, 12). In domestic animals isolated cases have been described in the dog (5), cat (10) and cattle (lit. see 5). In zoo mammals, one case has been described in a male Indian rhinoceros (6). One was in a (laboratory) cynomolgus monkey (Macaca fascicularis) (4). In ectotherms, 14 cases of neuroblastomas in fish are listed in the records of the RTLA (7). Separate descriptions of neuroepithelial tumours concern the coalfish (Pollachius virens L) (2) and the bream (Abramis brama) (14). In amphibians, a neuroepithelioma has been described in the axolotl (Siredon mexicanum) (1).
Experimentally, tumours of the nasal olfactory region have been provoked by s.c. injections of nitrosamine compounds (11).

To the best of our knowledge, there are no published records of such tumours in reptiles. One of our authors possesses a record of an unpublished case in a 16 foot Burmese python (7).

This paper aims at contributing to the knowledge of tumours in reptiles.

**Material and Methods**

An adult male blue-tongued skink (*Tiliqua scincoides*) weighing 0.8 kg, aged 4-5 years, had been submitted for clinical examination. Based on the clinical development the animal was euthanised. The head was fixed in toto in AFA fixative. Decalcification was with equal volumes of formic acid 36.8 % and sodium-formiate 6.8 %. The facial part of the head was divided in 5 sections. Materials were embedded in paraffin, cut at 5 µm and stained with Haematoxylin and Eosin (H&E).

**Results**

**Clinical history**

The owner related that there was some irritation of the nose, especially the right nasal duct was affected. At this side, the eye was wet. A wound had been noticed in the palate. A preliminary diagnosis of infection was made. enrofloxacin (Baytril®) and neo-bacitracine were prescribed. The animal was presented again, 17 days later. At that moment the animal sneezed and small blood stained specks were produced. Further examination revealed a proliferation in the rostral part of the palate. The possibility of a tumorous growth was considered. The therapy was continued and the owner was advised to present the patient in another ten days. At that moment a distinct improvement seemed apparent. Signs of infection had diminished and blood was no longer produced. However, the process in the palate was still present. Once more therapy was continued. Taking a biopsy was discussed with the owner. After another 10 days the swelling in the palate had grown and some blood was running from the right nostril. An explorative surgery was proposed.

The patient was anaesthetised using Ketamine HCl. Right at the first moment, part of the tumour broke away and blood spouted from the surface. It appeared that the right nasal passage was completely blocked by proliferated tissue. The right eye protruded. The bleeding could not be stopped and it was decided to euthanise the patient, using T61® (Bayer).

**Post mortem**

The animal was in good condition. The liver was pale in appearance but not distinctly abnormal. All other abdominal organs were normal.

At dividing the facial part in 5 sections, a proliferating process was seen in the right nasal canal. This occupied the nasal cavity up to the right eye. Near to the eye, in the caudal part of the nasal cavity an accumulation of non-structural material was present. The material filling the nasal cavity also bulged into the oral cavity.

At histology it was recognised that the most compact part of the process was at the level of the right Jacobson's organ.

The left Jacobson's organ was intact. This consisted of a cartilaginous center. It was surrounded by a thin layer of well-vascularised connective tissue and covered with pseudostratified olfactory epithelium. The apical surface of the epithelial cells had a brush border. The concave surface of the Jacobson's organ was provided with the characteristic, very high pseudostratified olfactory epithelium.

Extensive changes surrounded the right Jacobson's organ. The cartilaginous center was intact. Only on the ventral side, some small areas of normal olfactory epithelium were present. In these areas the subepithelial connective tissue was identical to that on the left side, with open bloodvessels.

Elsewhere, the surrounding connective tissue appeared compressed, as was concluded from the compacted arrangement of connective tissue fibers, in which blood vessels could not be recognised. This area was mainly covered with a flattened, atrophied epithelium. About 1/4 of the
total circumference of the cartilaginous center, was covered with tumour cells. In part these cells were continuous with the mass of the tumour. The tumour invaded in the surrounding connective tissue with blunt protrusions. It even progressed into marrow cavities. The free surface of the tumour protruded through the palatine slit. Especially towards the caudal extension, the tumour proliferated with slender finger like projections. On cross section, these projections revealed a large, vein-like central bloodvessel, some stroma and a single layer of tumour cells (pseudorosettes). It was in this area that the projections were surrounded by a mass of fresh blood. The tumour cells were slightly variable, depending on the location. In the compact areas, they were polymorph, and short. The nuclei were somewhat variable in size, but mainly slightly larger than in the intact epithelium of the left side. The nuclear chromatin was arranged in a normal pattern and with normal density. Mitotic figures were abundant. Homer Wright rosettes with a central space had been produced. These rosettes were formed by tall columnar to trapezoidal cells provided with a brush border and eccentric nuclei. The epithelial cells were broader, and as high as these covering the cartilaginous center of the intact (left sided) Jacobson's organ. Pseudorosettes had a similar epithelium. Along its extension in the caudal parts of the nose, the original respiratory epithelium had locally been lost. In these areas some unspecific reaction occurred. There was some invasion of lymphocytes in the submucosa and at one site the connective tissue near a spicule of bone was loosely structured, while along the periosteum the osteoblasts were activated. Subepithelial nerves were seen in the more caudal parts of the nose and were intact. A final diagnosis of olfactory neuroblastoma (esthesioneuroepithelioma) was made.

Discussion

The clinical symptoms can be fully explained from the pathologic findings. The marked bleeding must have been a result of damage to the well vascularised extensions of the tumour. The localisation of the tumour is characteristic of an olfactory neuroblastoma (3, 12). A unique aspect is the involvement of the Jacobson’s organ. Judging the aspect of the affected organ where next to normal and atrophic epithelium also nests of tumour cells have been found that were continuous with the central mass of the tumour, it might be suspected that this could be the origin.

Olfactory neuroblastoma is a more general name for tumours originating from the neur ectoderm (13). Depending on their differentiation, they are indicated asesthesioneuroepithelioma, esthesioneurocytoma or esthesioneuroblastoma (8). The presence of rosettes is characteristic of the classical olfactory neuroblastoma (8). The case of the blue-tongued skink illustrates that neuroepitheliomas occur in all classes of vertebr ae. In the case of the blue-tongued skink, the degree of differentiation almost reached this of the normal olfactory epithelium. Thus, the addition esthesioneuroepithelioma is a more refined designation of this tumour.

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LOW GRADE FIBROSARCOMAS IN GREEN TURTLES; IS FIBROPAPILLOMATOSIS GOING AMUCK?

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Key words: green turtle, fibropapilloma, fibrosarcoma, Hawaiian Islands

Abstract

The green turtle (Chelonia mydas) is protected under the U.S. Endangered Species Act and the Wildlife Laws of the State of Hawaii. Fibropapillomatosis (FP) is a disease of marine turtles characterised by multiple cutaneous masses ranging from 0.1 to more than 30 cm in diameter that has primarily affected green turtles. The disease has a worldwide distribution and has been observed in all major oceans and all species of marine turtles that are considered endangered of extinction. Where present, prevalence of the disease varies among locations, ranging from as low as 1% to as high as 90%. Although several viruses have been identified associated with the tumours, including herpesviruses, a retrovirus and a papilloma-like virus, the primary etiological agent remains to be isolated and identified. Concurrent infections of FP and cardiovascular trematodiasis have been recognised as the most important mortality factors of Hawaiian green turtles considerably reducing the survival of the species. The neoplastic processes previously observed by our previous studies and more recently during gross and histopathologic examination of 14 turtles collected in the Hawaiian Islands with FP suggested a synergistic effect of cardiovascular trematodes and the primary agent of FP.

Tumours in the internal organs of some turtles were characteristic of fibropapillomas, fibromas, myxomas, and low-grade fibrosarcomas. This study suggested that when occurring together, spiorchidiasis and GTFP represent a debilitating and fatal syndrome of Hawaiian green turtles. We describe the histopathology of cutaneous and internal spindle cell tumours found in green turtles from the Hawaiian Islands, and present histopathological and molecular evidence of the presence of low-grade fibrosarcomas. Histologically, some tumours of the nasopharynx, mouth and temporomandibular tissues appear to have an aggressive, invasive behaviour. These masses are well demarcated from adjacent tissues but demonstrate infiltration of surrounding stroma and bone lysis. Although there is no evidence of vascular invasion or high mitotic activity, these tumours have been classified as low grade fibrosarcomas.

The precancer to cancer sequence in the progression of benign to malignant tumours has been documented in other species with similar tumours, e.g. papillomas transforming into squamous cell carcinomas. Although there is no evidence of vascular invasion or high mitotic activity, further research is necessary to demonstrate whether the visceral lesions are the result of metastasised external papillomas, or indeed are multiple independent processes. We are trying to establish the biological behaviour and molecular characterisation of these tumours. Current retrospective and prospective studies are in progress to determine the implications of these novel findings.
Zusammenfassung

TUMORS OF THE CARAPACE OF PHILIPPINE GREEN SEA TURTLE (Chelonia mydas)

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Abstract

Ten tissue samples of fibropapilloma on the carapace of ten individual turtles were subjected to tissue processing and stained with Hematoxylin and Eosin, Masson’s Trichrome, and Alcian blue. Based on the tissue sections examined, all samples showed structures resembling the mammalian skin. In the epidermis, there was moderate to severe hyperplasia comprising of about 10-25 layers of epidermal cells. Orthokeratotic hyperkeratosis with hypertrophy of epithelial cells was present. There were multifocal areas of vacuolisation in the stratum granulosum down to stratum basale. There were focal areas of necrosis in the stratum basale.

The epidermis rests on a stroma resembling the dermis, which contains small blood vessels and well-differentiated fibroblasts haphazardly, arranged in interlacing bundles of collagen. In the papillary layer of the dermis, increase in downward growth of epidermal hyperplasia with regular to irregular pattern of dermal papillae was observed forming the rete ridges. The reticular layer of the dermis contains more abundant and thicker well-differentiated fibroblasts than the papillary layer. Few mitotic figures and no signs of malignancy or anaplastic changes were observed.

Key words: fibropapilloma, green sea turtle, Chelonia mydas

Introduction

Sea turtles are now designated as one of the threatened and endangered species, and among the species of concern are the green turtles Chelonia mydas. The presence of large cutaneous tumours can interfere with the turtles’ locomotion, vision, and breathing. On the other hand, visceral tumours can be locally invasive and affect organ function (7). Fibropapillomatosis in turtles was first documented in 1958 as a rare occurrence in Kaneohe Bay, Oahu in Hawaii. The prevalence of afflicted turtles at this location has been severe for the past ten years and continues in the present (2).

Fibropapillomatosis affects sea turtle populations worldwide, sometimes in epizootic proportions (9). It is characterised by the formation of lobulated fibrous tumours on all skin surfaces in the eyes, oral cavity, carapace, and less frequently on the internal organs (3, 5).

In the Philippines, cases of fibropapilloma-like tumours commonly located on the carapace were observed in the past decade. Considering the possible high prevalence of this disease in green turtles, which are near extinction, this study will help in establishing database on the nature and type of neoplasms in green turtles specifically those foraging in Baguan Island marine Turtle Sanctuary. This study also provided sources of information of histologically confirmed cases for epidemiological studies and may serve as guideline for preventive management of turtles in the future.
Materials and Methods

Tumour specimens were collected from turtles foraging in Baguan Island Marine Turtle Sanctuary in Tawi-Tawi, Philippines, during nesting season (July to September 1997). Turtles were properly restrained using ropes tied on the four limbs. Incision biopsies were performed for collecting tumour samples. Tumour samples, which measured 0.5 to 1 cm. in thickness, were fixed intact with 10% formalin. For larger masses, only representative portions from the central, middle and outer layers were collected and fixed. The volume of fixative was ten times that of the tissue to be fixed. All tissue sections were stained with hematoxylin and eosin. Representative sections from each specimen were stained with Masson’s trichrome and Alcian Blue as differential stains.

Results and Discussion

Based on tissue microscopic examinations, all samples showed structures resembling that of the different layers of the skin in mammals, namely the epidermis and the dermis comprising the stroma of the tumour. In the epidermal layer of the tumour, there is moderate to severe hyperplasia comprising about 10 to 25 layers of epidermal cells. The cells in the stratum corneum are non-nucleated with marked hyperplasia, also known as orthokeratotic hyperkeratosis. In comparison with the study made by Jacobson et al. (4), in the normal green turtle skin there was a relatively thin epidermis. This thin layer is composed of stratified squamous epithelial cells, ranging from four to seven layers in thickness. Epithelial cells were flattened at the surface, their nuclei become pyknotic and were lost and the cells were covered by a layer of “keratin-like” material which was approximately one half to two thirds the thickness of the cellular layer. However, similar lesions observed in this study such as orthokeratotic hyperkeratosis was also observed in all studies of fibropapilloma in green turtles (1, 4, 6). The degree of epithelial hyperplasia varies from mild to moderate (7-15 cells thick) on some conjunctival and palpebral muscles.

There is hypertrophy of the epithelial cells in the stratum corneum, which was also observed by Jacobson et al. (4). Two of the tumour samples showed papillated epidermal hyperplasia. Similarly, these fibropapillomas with extensive epithelial hyperplasia which often exhibit anatomising rete ridges or rete pegs extending deep down into the dermis was reported by Jacobson et al. (4).

All ten tissue sections from different individual turtles showed multifocal areas of intracytoplasmic vacuolation in the stratum granulosum down to stratum basale, similar to the observations of Jacobson et al. (4), and Aguirre et al. (1), which were described as multifocal areas of ballooning degeneration or vacuolation in the stratum basale and interpreted as the earliest lesions of tumour.

During the course of this study, no bacteria, fungi, algae, and mites were observed similar to the studies previously reported. Bacteria were not seen within intact green turtle fibropapilloma lesions and little inflammation was observed within tumours unless the surface is ulcerated. On the contrary some authors reported that the surfaces of tumours were colonised by a variety of bacteria, fungi, algae, and invertebrates including mites. In addition, no parasite eggs were observed compared to the early studies on green turtle fibropapillomabitis reported by Smith and Coates (8) who found trematode eggs within fibropapilloma, although parasites were not implicated then as the immediate cause of the disease. They later found papillomatous hyperplasia to be associated with the Digenean *Rhatydodoi des similes* in the gall bladder of green turtle.
The epidermis rest on a stoma resembling the dermis, which contains small blood vessels but no perivascular cuffing in all tissue samples, was observed. Fibroblasts are haphazardly arranged in interlacing bundles of collagen fibers. Similarly in other reports of fibropapilloma in green turtle, the papillary layer of the dermis consisted of numerous fibroblasts and compact collagen fibers, however, perivascular cuffs of lymphocytes and plasma cells were seen throughout the dermis.

Histochemical examination revealed presence of various amounts of collagen, which appeared blue in colour with Masson’s Trichrome, distributed all throughout the stroma. Thinner mucopolysaccharide appeared light blue with Alcian blue stains, was also present in the stroma of the tissue sections in all tumour samples. Collagen and mucopolysaccharide comprised the ground substance of the tumours examined, which are interspersed with the fibroblasts.

References
