The immunological, biochemical and molecular bases of canine senescence and carcinogenesis: a review

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ABSTRACT: Senescence is a complex set of processes involving several biochemical, molecular and metabolomic changes, including also many disturbances in the immunological system. There are many factors, described as intrinsic and extrinsic (environmental), that may lead to advanced body senescence. In this review, several of the biochemical as well as molecular factors involved in senescence are described. The importance of immunological deficiencies as well as changes in the immunological response after induction of senescence is also highlighted. Furthermore, the molecular basis of canine carcinogenesis in relation to interleukin expression and activation as well as the role of CD leukocyte common antigen in the identification of cancer development and progression, are also described.

Keywords: senescence; carcinogenesis; canine

List of abbreviations

4-HNE = 4-hydroxynonenal, **Aβ1**-42 = **β-amyloid** form, **AD** = Alzheimer disease, **alpha(1)AG** = alpha 1 acid glycoprotein, **bcl-2** = B-cell leukemia 2, **BER** = base-excision repair, **BRCA1** = breast cancer susceptibility gene-1, **CD** = cluster of differentiation, coating the surface of B lymphocytes and T lymphocytes, **CTVT** = canine transmissible venereal tumour, **FOXP3** = forkhead box P3, scurfin, **IFN-gamma** = interferon-gamma, **IL** = interleukin, **IVL** = intravascular lymphoma, **MGT's** = mammary gland tumours, **MHC** = major histocompatibility complex, **NFTs** = neurofibrillary tangles, **NHL** = non-Hodgkin's lymphoma, **NK** = natural killer, **OGG1** = 8-oxoguanine glycosylase 1, OSA = osteosarcoma, **PAC-1** = first procaspase activating compound, **PBLs** = peripheral blood lymphocytes, **pIL** = interleukin plasmids, **SLC1A3** = solute carrier family 1 (glial high affinity glutamate transporter), member 3, **SOD** = superoxide dismutase, **TCR** = T-cell receptor, **TCRBCL** = T-cell rich B-cell lymphoma, **TGF-beta** = transforming growth factor beta, **TIL's** = tumour-infiltrating lymphocytes, **Tregs** = T regulatory cells, **VEGF** = vascular endothelial growth factor

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1. Theories of body senescence vs. neoplastic transformation

Aging is the process whereby an organism accumulates changes over its lifespan. Even though there is a great increase in the incidence of disease in this period, especially neoplasms, this process is still poorly understood. Many hypotheses explaining the aging process, particularly based on molecular biology theories, have been advanced so far, including telomere shortening, epigenetic changes during the transcription process, DNA damage and increased mutation rate in somatic cells (Goldston 1995; Smith and Daniel 2012).

On the other hand aging can be considered a multifactorial process resulting from interactions between genetic factors and the environment, which is strongly influenced by the style of life (Pizza et al. 2011).

The wide range of neoplasms occurring in companion animals, mainly in canines, can serve as a unique model for the investigation of the neoplasm biology and studies on potential therapeutic opportunities. Because of its numerous physiologic and genetic similarities to the human, the domestic dog *(Canis familiaris)* is an important model for the aging process and neoplasm studies (Ahn et al. 2011; Kempisty et al. 2011). The clinical and biological similarities between naturally occurring human and canine neoplastic processes are difficult to reproduce in other animal models. The increase in scientific veterinary interest in animal aging and neoplastic processes and the large number of publications on the topic make the transfer of valuable insights much easier. What is more, the possibility to apply the tested therapeutic methods in humans is of great importance for transplantology (Pinho et al. 2012).

Several aging theories can be found in the literature, such as the genetic theory, evolution theory, free radical theory or immunologic theory of aging (Banks 1981; Sheffy and Williams 1981; Kearns et al. 1999; Serafini 2000; Kempisty et al. 2011). Aging is related to a significant increase in the occurence of diseases, including many types of neoplasms. Because of the complex and multifactorial nature of aging and neoplasia a thorough analysis of the relationship between these two processes still constitutes a significant challenge for researchers.

2. The biochemical and molecular basis of canine senescence and oncogenesis

Ahn et al. (2011) found 4-HNE, SOD1 and SOD2 to be present in the cervical and lumbar spinal cords of 10 to 12-year-old but not two to three-year-old dogs. They reported that the numbers of 4-HNE-, SOD1- and SOD2-immunoreactive cells was significantly higher in both the cervical and lumbar spinal cord of the older dogs and suggested a role for these proteins in generating DNA damage in neurons. The report of Borras et al. (2000) suggested that neuronal nuclear DNA fragmentation is an age-related phenomenon in dogs, not due to apoptosis, and occurs whenever certain factors render neuronal DNA more susceptible to autolytic fragmentation. Simultaneously, canines with oxidative changes in the brain provide a spontaneous model for studying early changes and their interrelationships in normal aging and in human neurodegenerative diseases (e.g. AD).

Alzheimer's disease is one of the major neurodegenerative diseases, which leads to a decline in cognitive function as a result of senile β-amyloid (Aβ1-42 form) plaque formation and the generation of neurofibrillary tangles (NFTs) in the brain (Papaioannou et al. 2001). Data from the literature indicate that Aβ1-42 as well as Aβ1-40 form in the plaques of the canine brain. It has been reported that the senile plaques are usually larger than those observed in the human AD brain and that the degenerative process in aging dog does not involve NFT formation (Dimakopoulos and Mayer 2002). Moreover, it was shown that in the degenerated canine brain the same plaque subtypes exist as in humans albeit not in the same regions (Dimakopoulos and Mayer 2002), and that they also may induce a decline in cognitive function in the aging dog. Salvin et al. (2010) have indicated that

canine cognitive dysfunction is a neurobehavioural syndrome affecting aged dogs while Azkona et al. (2009) have found that cognitive impairment shows a prevalence of 22.5% in geriatric dogs.

The main mechanism of repair of DNA damage due to base modification is base-excision repair (BER), which involves removal, using DNA glycosylases, of purine and pyrimidine bases that have been modified in a particular manner. Two distinct isoforms of human OGG1 proteins have been identified: α-OGG1 (alternatively named OGG1 type 1a), and β-OGG1 (type 2a). α-OGG1 mRNA is transcribed from exons 1 to 7, while β-OGG1 mRNA is transcribed from exons 1 to 6 plus exon 8 (Hirano 2008). In humans, the repair of oxidized guanine within DNA involves 8-oxoguanine glycosylase 1 (OGG1) (Dorszewska et al. 2009; Dezor et al. 2011). The inactivation of OGG1 DNA glycosylase in mouse leads to less severe inflammatory lesions (Touati et al. 2006) while mutations in the *OGG1* gene were found in human cancer (Hirano 2008) and in AD patients. In the study by Mao et al. (2007) it has been shown that the decreased excision activity of OGG1 in AD patients may be the effect of a mutation in the gene encoding *OGG1*. In that study, the presence of mutations was demonstrated in four of 14 AD patients; two of these involved C796 deletions which completely eliminated the activity of the OGG1 enzyme, and two were single point mutations, leading to decreased activity of the glycosylase. It is unavoidable that mutations may also affect other enzymes involved in the excision of 8-oxo2dG from DNA and lead to increased oxidative damage in the progression of AD and cancer.

Cancer is recognised as an age-related disease but the relationship between the two processes, aging and neoplasia, is not clear. It is known that the human incidence and mortality rates of most cancers increase with age up to 90 years, but they plateau and then decline with age. It is also known that cancer is the cause of 40% of all deaths in people aged 50 to 69 years and only 4% of deaths in centenarians (Vasto et al. 2009). Among the important questions related to the relationship between the aging process and cancer remains the influence of comorbidity on cancer outcome in the elderly, the problem of whether cancers are biologically similar or distinct as a function of different age, and the question of whether there exists an inherent resistance to cancer development (Ershler and Longo 1997). Recent studies have indicated that factors involved in the aging process may lead to the development of cancer, e.g., the generation and accumulation of cellular, genetic or epigenetic damage and increased levels of free radicals, or tumour suppressor genes, and a decline in growth hormone or sex steroids levels. On the other hand, some processes may exert different effects on aging and cancer, protecting from cancer but promoting aging, e.g., a restraint of cell proliferation. Simultaneously, it is known that mechanisms implicated in the prolongation of life span may be connected to an increased cancer risk. At the same time, it seems that a gentle balance between convergent and divergent mechanisms of aging and cancer could promote health during youth and adulthood and promote a healthier old age (Vasto et al. 2009).

Cancer may be considered a geriatric syndrome and the disciplines of gerontology and oncology have much in common. On the other hand, many similarities are found between human and canine cancers, including histologic appearance, tumour genetics, molecular targets, biological and clinical behaviour, and response to therapy (Peterson et al. 2010). The studies of Peterson et al. (2010) showed that in lymphoma, the most common cancer of humans and canines, evaluation of PAC-1 in canine lymphoma may provide important information for human cancer therapy. It is known that a small molecule of PAC-1, enhances procaspase-3 activity *in vitro*, and induces death in cancer cells by apoptosis thereby inhibiting antitumour activity (Peterson et al. 2009). However, high doses of PAC-1 induced neurotoxicity. In contrast to PAC-1, the similar molecule of S-PAC-1 did not induce neurotoxicity in mice or dogs and in canine cells induced partial tumour regression or stable disease and, thus, it may provide a novel cancer chemotherapeutic agent. On the other hand, the study of Bongiovanni et al. (2008) indicated that inhibition of cell death plays a key role in oncogenesis and tumour growth of some canine skin neoplasms. Simultaneously, it documented a direct correlation between lack of caspase-3 expression and a negative prognosis in human patients with nasopharyngeal carcinoma, associated with poor response to radiotherapy.

Murakami et al. (2008) showed that anti-apoptotic factors play a role in the malignant growth of canine vascular tumours, and malignant proliferation in this carcinoma was associated with bcl-2 and survivin expression. However, the studies of Al-Dissi et al. (2010) in canine simple mammary gland adenocarcinomas have shown that vascular endothelial growth factor (VEGF) may stimulate

tumour cell proliferation through an autocrine loop, and a change in VEDF levels may lead to malignancy of canine cancer.

It seems that cellular senescence and malignant transformation in canine tissues are common. However, in geriatric dogs biochemical changes may lead to the development of canine cancer with a distinct resistance to therapy.

3. The process of senescence and its links to changes in the immune system

Senescence is not a disease but involves a complex set of genetic and biochemical changes which develop in a body of a specific age, and which may lead to increased susceptibility to selected diseases. An example can be provided by the immune system which, upon the organism reaching a specific age, manifests a reduced pro-inflammatory response. This decreased response of the system was shown to be not necessarily linked to an increased risk of tumour development. Studies on animal models demonstrated that the process of senescence in the immune system can paradoxically be linked to a reduced tumour growth rate and a reduced metastatic ability in several types of tumours. On the other hand, much clinical and epidemiological data point to a strict relationship between chronic infections, inflammatory conditions and the development of tumours (Coussens and Werb 2002; Schacter and Weitzman 2002). The principal element of a pro-inflammatory response in a tumour is suggested to involve macrophages, which provide increased amounts of growth factors for epithelial and endothelial cells, including pro-inflammatory cytokines and chemokines, which modify angiogenesis (Allavena et al. 2008). These factors also stimulate the proliferation and progression of neoplastic cells. Moreover, several mediators released by inflammatory or neoplastic cells and which inhibit the function of the immune system, decrease the antineoplastic response and, thus, promote tumours progression (Mantovani et al. 2008; Sica et al. 2008).

4. The role of interleukin in oncogenesis and cancer therapy in dogs

Over the last 10 years many reports have detailed investigations into the role of interleukins or other immune-specific proteins in oncogenesis, prevention and cancer therapy. The function of these molecules in human cancer is well recognised, although still little is known about the role of immunological response status in canine cancer development. In most cases dogs are used as a model for human cancer therapy, for the cloning of nucleic acids as well as for xenograft studies. The most frequent canine cancers used as a model for human cancers include haemangiosarcomas, lymphoid and hematopoietic malignances or mammary gland tumours (MGT's). Akhtar et al. (2004) investigated the factors that may inhibit angiogenesis in malignancy using the canine haemangiosarcoma cell line, derived from malignant endothelial cells. They used a haemangiosarcoma biopsy, which was engrafted in a bg/nu/XID mouse to expand the tumour cells *in vivo*. The specific SB-HAS cell line, which was derived from xenografts, expressed vascular endothelial growth factor receptors (VEGF1, VEGF2), CD31, CD146 as well as integrins and interleukin (IL)-8. All of these investigated proteins stimulate endothelial cell growth. In this study they also determined the immunostimulatory and antiangiogenic effects of IL-12, which suppress angiogenesis and tumour growth. Generally, using the SB-HAS xenograft cell line model to determine the role of both IL-8 and IL-12 interleukins as anti-angiogenic factors which prevent tumour growth may be an important tool in cancer therapy. Similar investigations related to the dog cancer model and tumour therapy were conducted by Dickerson et al. (2002). They used lymphohaematopoetic malignances as common spontaneous diseases in dogs which are closely related in their biological behaviour as well as response to therapy to those in humans. They determined the expression pattern of the interleukin 2 receptor (IL-2R) in 13 dogs with multicentric non-Hodgkin's lymphoma (NHL) and six dogs with leukaemia. The molecular study included the cloning and sequencing analysis of the complete coding domains of the wild-type canine IL-2R, including alpha, beta and gamma subunits. All of these subunits of IL-2R were expressed at the mRNA level in a dog with B-cell acute lymphocytic leukaemia and with acute monoblastic leukaemia. In B-cell leukaemia and T-cell leukaemia only the beta and gamma subunits were expressed. Similarly to the previously reported data the results indicated that IL-2R is specifically expressed in dog lymphohematopoietic malignancies. Moreover, the similarity to human cancer makes it possible to use this gene

and protein in anticancer therapy and this could be of interest in the treatment of human haemolymphatic malignancies.

Canine hematopoietic cancers are characterised by several disturbances and abnormalities in blood-specific cell counts. Moreover, the immunological status and expression of specific molecules which modulate the immune response, such as IL-6, TGF-beta or alpha 1-acid glycoprotein (alpha(1)AG), may be also used as a potent diagnostic marker of these malignancies. Itoh et al. (2009) investigated differences in the immunological status of dogs with cancers at different stages of development in comparison to normal, healthy animals in relation to the expression of these markers. They showed that the number of leukocytes in the whole blood was higher in dogs with cancer as compared to normal dogs, and this count increased in the more advanced tumours. In the tumour-bearing dogs the number of inflammatory cells, such as neutrophils, acidophils and monocytes, was also higher. In contrast, the number of CD4(+) T-cells was lower than in healthy dogs as was the lymphocyte count and the low number of these cells correlated with tumour progression stage. The cancer-specific expression of molecules such as IL-6, TGF-beta and (alpha(1)AG) was also increased in tumour-bearing dogs. All of the three investigated molecules might be used as prognostic markers for the evaluation of cancer development and progression. Measuring the specific expression pattern of these proteins might also be useful in relation to the low anti-tumour immunity in such dogs.

One of the most frequent cancers which develop in canines is the mammary gland tumour (MGT). This cancer is characterised by higher numbers of tumour-infiltrating lymphocytes (TIL's) as well as by local cytokine production. All of these factors strongly influenced tumour growth and progression. The role of IL-1 and IL-6 in canine cancer development was previously described. However, still little is known about the role of these proteins in cancer metastasis and tumour progression. Kim et al. (2010) investigated the role of these proteins and breast cancer susceptibility gene-1 (BRCA1) in canine MGT's. They found a pronounced correlation between IL-1 and IL-6 expression and MGT metastases. An association between the expression of TIL's, cytokines and mutations in the BRCA1 gene was also noted. In conclusion, the expression of the investigated cytokines as well as mutational analysis of BRCA1 and lymphocyte tumour specific infiltration may be used as markers of tumour progression in relation to possible metastases.

Pulmonary metastasis is the most common reason of death in osteosarcoma. In recent years pet osteosarcoma has been used as a potential model in studies on paediatric cancer development and progression. Although the mechanisms of osteosarcoma development in humans as well as in canines are well recognised, there is still little known about the function of possible factors in the development of metastases and drug resistance of this aggressive paediatric cancer. Pet dogs naturally develop osteosarcoma and, therefore, it may provide a new important model for the analysis of several crucial factors linked to expression patterns in human cancer if not for the analysis of human cancer development in general. Paoloni et al. (2009), using parallel oligonucleotide array platforms, identified two genes, IL-8 and solute carrier family 1 (glial high affinity glutamate transporter), member 3 (SLC1A3), which were specifically expressed in dogs with osteosarcoma but not in all paediatric osteosarcoma patients. The expression of these two proteins in human and canine osteosarcoma provides constitutes possible new markers responsible for specific cancer development in this species as well as in humans. Osteosarcoma is another type of invasive and aggressive cancer the molecular and biological behavioural traits of which are conserved between humans and dogs. Several common features provide new opportunities to understand the role of cytokines (especially interleukins) in cancer development, progression and metastases as well as improving our understanding of cancer biology and highlighting possible efficient and appropriate therapies.

Interleukins are well recognised as markers for the development, progression and metastasis of several different types of cancer in humans as well as in canines, as was mentioned above. Moreover, the interleukins are also targets for cancer specific immunotherapy in several types of tumours, including canine transmissible venereal tumour (CTVT) or pulmonary metastases. CVTV is characterised by decreased MHC antigen expression and is used as a model for investigations on the interaction between immunity and cancer cells. High concentrations of TGF-beta, which is synthesised by CVTV cells, disturb the host immune response and, therefore, lead to cancer growth and progression. One of the mechanisms which inhibit this process in later stages of tumour growth involves the synthesis of IL-6, the cytokine which antagonizes TGF-beta and leads to

an increased IFN-gamma activity, which promotes MHC expression. Chou et al. (2009) used a combinatory treatment with IL-6 and IL-15 plasmids (pIL-6/pIL-15) in the therapy of CVTV-bearing beagles. IL-6 was the cytokine that was used as an inhibitor of TGF-beta protein while IL-15 activates NK-specific cytotoxicity. The intratumour plasmid delivery was performed by electroporation. After the treatment, MHC antigen expression on CVTV cells increased from the level of 5.9% to 34%. The number of CD8(+) T-cells which infiltrated the tumour increased also four-fold. These results suggest that use of plasmid (pIL-6/pIL-15) specific therapy leads to an increased immune response and may represent a useful target for inducing tumour regression. Moreover, this is another instance which indicates that canine-specific interleukins may be a good prognostic tool in human cancer therapy.

Another way to use interleukins in cancer therapy involves the application of IL-2 liposomes in aerosol. The use of this strategy leads to biological activity and is safe for normal dogs. Khanna et al. (1997) used dogs with pulmonary metastases and with primary lung carcinoma as the model for treatment with an aerosol containing IL-2 liposomes. They demonstrated complete regression of metastasis in two of the four dogs with metastatic pulmonary osteosarcoma. For the first group of two dogs regression remained stable for more than 12 months and in the second group of four dogs the regression was stable for 20 months. The stabilisation of cancer development was recorded in one of two dogs with lung carcinoma for more than eight months. In the second dog from this group a progression of the cancer was noted. The primary lung carcinoma as well as pulmonary metastases represents another cancer type that may be treated using immune-targeted approaches based on interleukin therapy. These results demonstrated the specific features of dogs as well as pet dogs, which are convenient models for studying immunotherapy in cancer prevention and for investigating potential molecular targets to induce cancer regression-specific mechanisms.

5. The identification of canine CD leukocyte antigen family markers and their role in cancer development and diagnosis

The CD leukocyte antigen family proteins are used as markers of several human and animal diseases including cancers. They are most frequently used to identify cancers using flow cytometry, which is a useful tool for characterising the immunophenotype of neoplastic cells and allows for CD protein expression analysis. Cancers amenable to such analysis include lymphoma malignancies as well as several other carcinomas, such as osteosarcoma (OSA). For more than 10 years molecular investigations have identified several different types of CD antigens as markers of various types of human and canine lymphoma. The most important players in the development of this cancer type involve CD antigens, such as those of B, T and natural killer (NK) cells and their specific patterns of expression. The last type of tumours is characterised by proliferation in the group of lymphocytes lacking clonally distributed protein receptors, specific for the other lymphocytes types, of B- or T-cells. However, in several species of mammals including humans, CD8(+) peripheral blood lymphocytes (PBLs) exhibit NK cell activity. Although the NK cell subpopulation is well characterised in humans, there is still a lack of information about the activity of these specific cells and their potential role in the diagnosis of lymphoma in canine species. There are only a few recently published reports which identify a potential role for the CD8(+) subpopulation in NK cell activity in dogs. Lin et al. (2010) used CD8(+) cells derived from PBLs and lymphokine (IL-2)-activated killers (LAKs) of PBLs, which were CD3(+), CD4(–) CD21(–), CD5(lo), alpha/beta TCR (+) and gamma/delta TCR(–). They found an increased mRNA expression for NK cell-specific receptors, such as NKp30, NKp44, NKG2D and CD16, for PBLs and NKG2D and CD56 for LAKs, as compared to CD8(–) cells. The cytotoxic activity of NK cells was also higher in the population of CD8(+) cells as compared to CD8(–) cells. Moreover, these results proved the previously formulated hypothesis that the IL-2 stimulated CD8(+) lymphocyte subpopulation may display NK activity and cytotoxicity. These results showed a new feature of CD8(+) cells which may be exploited in the future in focusing investigations on several new pathways in the diagnosis of human and canine diseases, especially lymphomas.

Canine malignant lymphoma is the most common lymphoid tumour in this species; the immunophenotype has an important prognostic value in T-cell lymphoma, which has a worse prognosis than B-cell lymphoma. There are several described T- and B-cell specific markers, very helpful in the

identification of such cancers. The most important of these markers include the T-cell specific marker CD3 and B-cell specific marker pan-B-cell marker, CD79a. Milner et al. (1996) suggested that both CD3 and CD79a surface antigens displayed cross-reactivity across species in lines of B- and T-cell lymphoma. Below, the role of these antigens in the diagnosis of lymphoid cancer development is discussed with respect to CD-specific expression profile.

The other commonly expressed membrane antigens which are manifested on blood leukocytes and neoplastic cells include CD18 and CD45. These antigens are the most frequently employed markers in the identification of several types of lymphoid malignancies in the blood, as compared to healthy donors and patients with reactive diseases in humans and canines. Comazzi et al. (2006) investigated the role of the leukocyte antigen expression in dogs with precursor lymphoid malignancies and mature neoplasms (chronic lymphocytic leukaemia or lymphoma). Using flow cytometry they found a small granulocytic population (L/N ratio) in the neoplastic subjects for both CD18 and CD45. Neoplastic cells were characterised by a decreased L/N ratio for CD18 and Cd45 antigens, which may be explained as a less mature expression pattern than that of normal cells or as a decreased expression of these two antigens on the surface of a cellular subpopulation. The detectable differential expression of CD antigens in the neoplastic cell subpopulation may be explained by pseudo-clonality and the different activity of these cells.

The characterisation of B- and T-cell-specific antigen expression using flow cytometry is the most frequently used tool to assess generalised or multisystemic lymphoma. In several cases the lymph is used as a good prediction material to determine the stage of development or diagnosis in such types of cancers. In normal lymph nodes, the T-cell population exhibits the expression of several CD antigens, such as CD3, CD4 and CD8 beta. The B-cells express CD21 antigen or surface IgM (IgM). Wilkerson et al. (2005), using canine lymphoma/leukaemia as a model, identified a different expression pattern of CD molecules on the surface of B- and T-cells. They demonstrated CD79a, IgM, and CD21 expression on B-cells, whereas in the case of three B-cell populations they also detected CD34 antigen expression, a marker of stem cells. The T-cell lineage was identified by the expression of the combination of CD3(+), CD4(+) and CD8(–). Several cases displayed the mixed expression of B- and T-cell-specific antigens and in three cases CD14 antigen manifested an increased expression. The neoplastic cells were characterised by the expression of both B (CD79a, CD21) and T-cell (CD3)-specific antigens. These results confirmed the previously reported data reported above, that canine lymphoma or leukaemia is characterised by a differential expression of several antigens specific for both B- and T-cells and that, taking into consideration this fact, we can conclude that this type of canine cancer is highly heterogeneous in relation to counts of specific cell subpopulations as well as immunological characteristics. Moreover, the CD antigen expression profile may be also used as a potential marker in the diagnosis of human and canine lymphoid malignancies. Similar results were obtain by Aquino et al. (2000), who investigated the T-cell rich B-cell lymphoma (TCRBCL) in dogs and demonstrated that metastatic liver lesions were characterised by monomorphic large neoplastic lymphoid cells, which were similar to the large neoplastic cells and were CD3-negative as well as BLA.36-positive, which confirmed the hypothesis of the B-cells lineage. These results also proved the similarity between canine lymphoma and related human tumours.

The other type of rare canine angiotropic large-cell lymphoma is intravascular lymphoma (IVL), which is characterised by neoplastic lymphocytes proliferating within the lumina of blood vessels. The second feature of this cancer is the absence of a primary extravascular mass or leukaemia. McDonough et al. (2002) characterised the neoplastic cell-specific antigen expression, which resembled the above mentioned results. The neoplastic cells had a specific pattern of CD expression, presenting as T-cell specific (CD3+/IgG–/CD79a–), or B-cell specific (CD3–/CD79a.dim/IgG+) patterns. In six cases they identified a non-T- and non-B-cell pattern (CD3–/IgG–/CD79a–). These results confirmed the previously reported data on the specific expression pattern of CD antigens on neoplastic cells in canine lymphoma and hinted that canine lymphoma in relation to its CD profile is characterised by several features similar to those of human cancer and, therefore, may be used in future experiments as a model for cancer prevention as well as therapy. However, in contrast to human B-cell-specific lymphomas, the immunophenotype of canine cancer is highly heterogeneous; in this species IVL derive mostly from T-cells, non-B-cells as well as non-T-cells.

The identification of the CD antigen expression profile was also performed in several other types of cancer, such as canine OSA. In a routine cancer diagnosis several surface antigens, such as (CD4 or CD25) or proteins specific for the interior of the cell [forkhead box3 (FoxP3)] are mostly identified. However, no anti-canine CD25 antibody was defined and investigated. Therefore, Rissetto et al. (2010) using cloning and transfection of the canine CD25 gene into human HeLa cells produced canine anti-CD25 antibody and identified subsequently the antigen on the cell surface using flow cytometry. They showed a lower expression profile of CD4+/CD25+/FoxP3+ in T regulatory cells (Tregs) in the tumour draining lymph nodes in dogs with OSA, as compared to healthy control. They did not detect differences in the number of Tregs between the group with OSA and control animals. Thus, they argue that the canine CD25 antigen might constitute another marker for characterising Tregs cells in canine cases of OSA. However, they did not find any association between the CD25 expression profile and clinical variables in canines with OSA and, therefore, this study did not support any of suggested clinical applications of the antigen.

6. CONCLUSION

Summing up the above, we would like to draw the reader's attention to the clear relationship between neoplasia and the induction of inflammatory processes. Tumours characterised by a negligible synthesis of cytokines or chemokines are also marked by very restricted blood supply and slow growth. Specific pro-inflammatory stimulation is linked to an accelerated angiogenesis and synthesis of growth factors, which lead to an accelerated tumour growth. On the other hand, however, the pronounced inflammatory reaction associated with the production of a high number of infiltrating monocytes leads to cytotoxicity and tumour regression (Allavena et al. 2008). The processes of cell senescence and neoplasia share several common traits. Genetic studies in recent years have proven that changes in gene structure in the form of mutations (the number of which correlates with advancing age) may significantly affect neoplasia. Therefore, apart from the analysis of selected gene expression in tumours (using transcriptomic and proteomic techniques) attention should also be paid to structural alterations in the genome, in the form of mutations or polymorphisms.

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