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REVIEW

Clonally transmissible cancers in dogs and Tasmanian devils

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Tasmanian devil facial tumor disease (DFTD) and canine transmissible venereal tumor (CTVT) are the only known naturally occurring clonally transmissible cancers. These cancers are transmitted by the physical transfer of viable tumor cells that can be transplanted across histocompatibility barriers into unrelated hosts. Despite their common etiology, DFTD and CTVT have evolved independently and have unique life histories and host adaptations. DFTD is a recently emerged aggressive facial tumor that is threatening the Tasmanian devil with extinction. CTVT is a sexually transmitted tumor of dogs that has a worldwide distribution and that probably arose thousands of years ago. By contrasting the biology, molecular genetics and immunology of these two unusual cancers, I highlight the common and unique features of clonally transmissible cancers, and discuss the implications of clonally transmissible cancers for host-pathogen evolution.
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Introduction

Cancer arises when a single-cell lineage acquires somatic mutations that promote it toward a program of continued proliferation. Natural selection favors the most prolific subclones, often steering the cancer toward a more aggressive phenotype. By its nature, cancer is counterselective and often lethal to its host, and thus cancer is usually an ultimately short-lived and self-destructive entity.

This review focuses on the biology of two highly unusual cancers that have overcome the limitations of existing within a single host by gaining the ability to spread between individuals. Tasmanian devil facial tumor disease (DFTD) and canine transmissible venereal tumor (CTVT) are clonally transmissible cancers that are spread by the physical transfer of cancer cells between hosts. Thus these two cancers have continued to exist by serial transfer between hosts even long after the death of the individuals that gave rise to them. As such, these two diseases offer unique opportunities to study the biology of cancers that have embarked on new evolutionary trajectories as free-living infectious agents.

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Devil facial tumor disease is a facial tumor that affects Tasmanian devils, and CTVT is a venereal tumor of dogs. DFTD and CTVT are the only known naturally occurring clonally transmissible cancers, and although they share a number of common features, they have emerged independently and have strikingly different life histories. By comparing and contrasting DFTD and CTVT, I have attempted to elucidate some of the common and unique features of these two unusual cancers.

DFTD

Tasmanian devils (*Sarcophilus harrisii*) are the largest extant marsupial carnivore, and are endemic to the Australian island of Tasmania. Weighing between 7 and 11 kg, devils are solitary opportunistic nocturnal scavengers (Owen and Pemberton, 2005). DFTD is a recently emerged infectious disease characterized by the appearance of primary tumors on the face, neck and inside the mouth of affected animals, which frequently become very large (>3 cm diameter) and ulcerate (Figure 1; Hawkins *et al*., 2006; Loh *et al*., 2006a; Lachish *et al*., 2007). DFTD causes death within months of the appearance of initial symptoms and has caused widespread devil population decline (Hawkins *et al*., 2006; Lachish *et al*., 2007; McCallum *et al*., 2007). Indeed, if current rates of disease spread and population decline continue, DFTD poses a risk of extinction of wild devils within 25–35 years (Hawkins *et al*., 2006; Lachish *et al*., 2007; McCallum *et al*., 2007).

Lesions associated with DFTD were first observed in devils in northeastern Tasmania in 1996 (Hawkins *et al*., 2006). The first confirmed case was found in a similar area in 1997 (Loh *et al*., 2006a). The number of confirmed DFTD cases steadily rose from 1 in 1997 to 68 in 2004 (Loh *et al*., 2006a). By 2007, extrapolation based on location of confirmed cases indicated DFTD had occupied at least 59% of the area of mainland Tasmania (McCallum *et al*., 2007). The failure to find evidence for DFTD in records of more than 2000 devils observed at nine locations between 1964 and 1995, or in 174 archival devil specimens collected between 1941 and 1989, strongly suggests that DFTD, at least in its current form, emerged recently (Hawkins *et al*., 2006; Loh *et al*., 2006a).

Devil facial tumor disease is a soft tissue neoplasm characterized by pleomorphic round- to spindle-shaped cells (Loh *et al*., 2006a). Cytologically, DFTD cells appear as large centrally nucleated cells with few

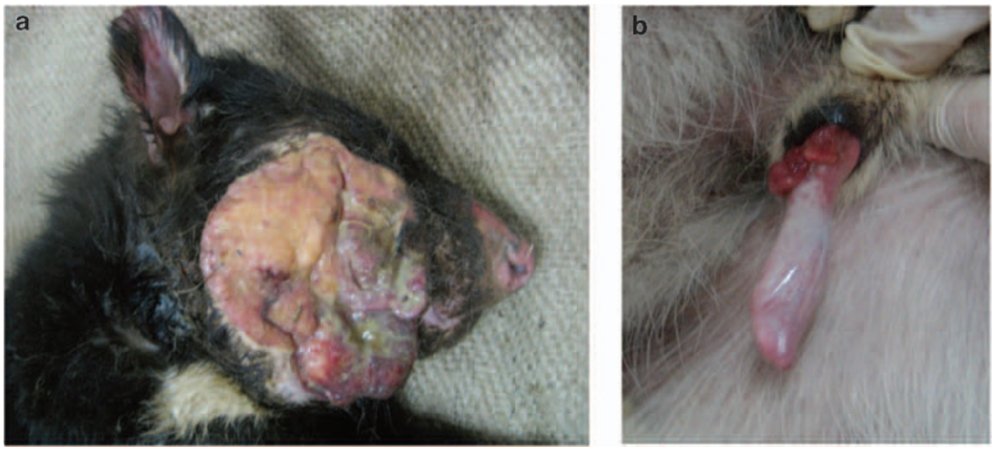

Figure 1 (**a**) Devil facial tumor disease (DFTD) and (**b**) canine transmissible venereal tumor (CTVT).

distinctive structural or ultrastructural features (Loh *et al*., 2006a). DFTD frequently metastasizes, particularly to regional lymph nodes and visceral organs (Loh *et al*., 2006a). Although undifferentiated in appearance, DFTD has been characterized histologically as a tumor of neuroendocrine origin due to its expression of vimentin, S100, Melan A, CgA, NSE and synaptophysin, and the absence of expression of epithelial, neural, endothelial and hematopoietic markers (Table 1; Loh *et al*., 2006a, b). Only 32% of DFTD cases showed evidence of immune cell infiltration (Loh *et al*., 2006b).

Clonal transmission was first proposed for DFTD based on cytogenetic evidence (Pearse and Swift, 2006). The constitutive diploid chromosome number of the Tasmanian devil is 14, including XX or XY sex chromosomes. Pearse and Swift (2006) found that 11 DFTD tumors collected from different locations in eastern Tasmania over a 12-month period had a karyotype of 13 chromosomes with identical rearrangements. The tumors were missing five chromosomes including both sex chromosomes, and had gained four unidentifiable marker chromosomes. Furthermore, one devil was found to have a constitutive chromosomal inversion that was absent in the DFTD derived from the same animal. The consistent and complex pattern of rearrangement in DFTD cells suggests that DFTD tumors are clonally transmitted as allografts that are genetically distinct from their hosts.

The clonal nature of DFTD was further confirmed by molecular genetic studies. Microsatellite alleles at four loci as well as alleles at fragments of several major histocompatibility complex (MHC) loci were identical in 15 DFTD tumors and in many cases were distinct from their hosts (Siddle *et al*., 2007a). The fact that all tumors shared an identical genotype that was genetically distinct from their hosts strongly suggests a monophyletic origin for DFTD.

Experimental transfer of DFTD cells or tissues to unaffected devils has also been performed, showing that DFTD can be transferred as an allograft (Pyecroft *et al*., 2007; Obendorf and McGlashan, 2008). Furthermore, electron microscopy studies of 29 DFTD tumors from 12 individuals failed to find any evidence of viral particles (Loh *et al*., 2006a; Pyecroft *et al*., 2007).

DFTD life cycle

Biting has been suggested as the most plausible route of DFTD transmission, although other modes of transmission, such as cannibalism of infected carcasses or sharing food, are possible (Hawkins *et al*., 2006; Pearse and Swift, 2006; Pyecroft *et al*., 2007; Hamede *et al*., 2008; Obendorf and McGlashan, 2008). Obendorf and McGlashan (2008) reported the recovery of DFTD cells from smears of canine teeth in direct contact with orally erupting DFTD tumors. In addition, two cases have been reported in which DFTD tumors developed from lesions apparently caused by bite wounds (Obendorf and McGlashan, 2008). Biting as the predominant route of transmission may also explain the apparent facial tropism of DFTD, as Hamede *et al*. (2008) observed that more than 85% of bites delivered during fighting at feeding interactions, as well as the majority of injuries in adults, occurred on the head. DFTD affects males and females equally, consistent with the observation of an equal number of injuries in males and females (Lachish *et al*., 2007, 2009; Hamede *et al*., 2008).

Devil facial tumor disease may have an incubation period of 6 months or more (Hawkins *et al*., 2006; Lachish *et al*., 2007, 2009; McCallum *et al*., 2007; Obendorf and McGlashan, 2008), but once overt signs of DFTD have appeared, the disease invariably progresses toward increased tumor volume (Hawkins *et al*., 2006; Coupland and Anthony, 2007). The longest period that a devil has been known to survive with DFTD in the wild is 9 months, with most devils surviving no more than 6 months (Hawkins *et al*., 2006; Lachish *et al*., 2007). Although in most cases of DFTD the cause of death is unknown, secondary infection, secondary complications of increasing tumor size or metastasis and starvation due to either obstruction of feeding or caloric diversion to the tumor have been suggested (Pearse and Swift, 2006; Pyecroft *et al*., 2007).

Table 1 Genes that have been studied in DFTD and CTVT

Gene symbol	*Gene name*	*Detection method*	*Details*	*Reference*
DFTD				
MHC class I	MHC class I	RT–PCR, sequencing, SSCP	6 unique classical class I sequences representing 5 loci expressed	Siddle *et al.* (2007a)
MHC class II	MHC class II	RT–PCR, sequencing, SSCP	4 unique class II DAB sequences representing at least 2 loci expressed	Siddle *et al.* (2007a)
MLANA	Melan-A	IHC	28% DFTD cases positive	Loh *et al.* (2006b)
VIM	Vimentin	IHC	100% DFTD cases positive	Loh *et al.* (2006b)
S100	S100 (several loci)	IHC	85% DFTD cases positive	Loh *et al.* (2006b)
SYP	Synaptophysin	IHC	97% DFTD cases positive	Loh *et al.* (2006b)
ENO2	Enolase (gamma, neuronal)	IHC	100% DFTD cases positive	Loh *et al.* (2006b)
CHGA	Chromogranin A (parathyroid secretory protein 1)	IHC	100% DFTD cases positive	Loh *et al.* (2006b)
CTVT				
MHC class I	MHC class I	RT–PCR, sequencing, IHC	Expressed in 0–4% of progressive CTVT cells, expressed in a 32–34% of regressive CTVT cells	Cohen *et al.* (1984); Epstein and Bennett (1974); Hsiao *et al.* (2002); Murgia *et al.* (2006); Yang *et al.* (1987)
MHC class II	MHC class II	RT–PCR, sequencing, IHC	Expressed in a 0–3% of progressive CTVT cells, expressed in ~37% of regressive CTVT cells; Alleles related to those found in wolves and huskies	Epstein and Bennett (1974); Hsiao *et al.* (2002); Mizuno *et al.* (1994); Murgia *et al.* (2006); Perez *et al.* (1998); Yang *et al.* (1987)
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	PCR, Southern blot, northern blot, sequencing	Genomic rearrangement involving a LINE element upstream of first exon; Expressed in CTVT; LINE element rearrangement may promote transcription	Amariglio *et al.* (1991); Choi and Kim (2002); Katzir *et al.* (1987); Katzir *et al.* (1985)
TP53	Tumor protein p53	Sequencing	T964C mutation present in CTVT cases from Korea; *T963C* mutation not present in CTVT cases from Mexico	Choi and Kim (2002); Vazquez-Mota *et al.* (2008)
RPPH1	Ribonuclease P RNA component H1	Sequencing	Polymorphic nuclear gene; CTVT sequence identical to dogs and wolves	Rebbeck *et al.* (2009)
G6PD	Glucose-6-phosphate dehydrogenase	IHC	Activity present	Hernandez-Jauregui *et al.* (1973)
VIM	Vimentin	IHC	100% CTVT cases positive	Gimeno *et al.* (1995); Marchal *et al.* (1997); Mozos *et al.* (1996); Mukaratirwa *et al.* (2004); Sandusky *et al.* (1987)
TNC	Tenascin C	IHC	CTVT cells positive in 0% progressive, 50% regressive CTVTs	Mukaratirwa *et al.* (2004)
TERT	Telomerase	TRAP assay	80% CTVTs (progressive and regressive phase) positive for telomerase activity	Chu *et al.* (2001a)
PCNA	Proliferating cell nuclear antigen	IHC	45% cells positive in progressive CTVT; 36% cells positive in regressive CTVT	Chu *et al.* (2001a)
MKI67	Antigen identified by monoclonal antibody Ki-67	IHC	Expressed in a high proportion of tumor cells in progressive phase; expressed in a small proportion of tumor cells after chemotherapy-induced regression	Gonzalez *et al.* (2000)
HSPD1	Heat shock 60 kDa protein 1 (chaperonin)	Western blot, IHC	Higher in regressing than progressing CTVT; Higher in CTVT than in normal tissue	Chu *et al.* (2001b)
HSP70	Heat shock protein 70	Western blot, IHC	Higher in CTVT than in normal tissue	Chu *et al.* (2001b)

Table 1 Continued

Gene symbol	*Gene name*	*Detection method*	*Details*	*Reference*
HSP90	Heat shock protein 90	Western blot, IHC	Positive in CTVT	Chu *et al.* (2001b)
TGFB1	Transforming growth factor-β1	RT–PCR, western blot, IHC, ELISA, activity blocked by antibody	Expressed in the majority of cells in progressive and regressive CTVT	Hsiao *et al.* (2008); Hsiao *et al.* (2004)
STAT1	Signal transducer and activator of transcription 1 (91 kDa)	Western blot	Phospho-Tyr-701 induced by IL6/IFNγ and present at higher levels in progressive than regressive CTVT	Hsiao *et al.* (2008)
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	Western blot	Phospho-Tyr-705 induced by IL6 and IL6/IFNγ and present at higher levels in progressive than regressive CTVT	Hsiao *et al.* (2008)
CREB1	cAMP responsive element-binding protein 1	TranSignal Protein/DNA Array	Induced in CTVT cells by IL6, IFNγ and IL6/IFNγ	Hsiao *et al.* (2008)
IRF1	Interferon regulatory factor 1	TranSignal Protein/DNA Array	Induced in CTVT cells by IL6, IFNγ and IL6/IFNγ	Hsiao *et al.* (2008)
NFκB1	Nuclear factor of κ-light polypeptide gene enhancer in B cells 1	TranSignal Protein/DNA Array	Induced in CTVT cells by IFNγ and IL6/IFNγ	Hsiao *et al.* (2008)

Abbreviations: CTVT, canine transmissible venereal tumor; DFTD, devil facial tumor disease; IHC, immunohistochemistry; MHC, major histocompatibility complex; RT–PCR, reverse transcriptase PCR; SSCP, single-strand conformation polymorphism; TRAP, telomere repeat amplification protocol.

The persistence of DFTD within one devil population whose density has decreased by more than 95% suggests that DFTD transmission may be independent of population density (Lachish *et al.*, 2007). Indeed, it has been suggested that the dynamics of DFTD transmission may be similar to a sexually transmissible disease, as DFTD prevalence may fluctuate seasonally, most injuries to adults are inflicted during mating season, and DFTD is rare in juveniles that are not sexually mature (Hawkins *et al.*, 2006; Lachish *et al.*, 2007; Hamede *et al.*, 2008; McCallum, 2008). However, Hamede *et al.* (2008) found that frequency of bites increased with increasing devil density during feeding interactions, suggesting that population density may affect probability of transmission. In addition, Hamede *et al.* (2008) showed that subadults, most of which would presumably not yet be sexually active, also sustained injuries, suggesting opportunities for DFTD transmission in immature individuals. Determining the transmission dynamics of DFTD will have important implications for devil conservation, as single-host diseases transmitted in a frequency-dependent manner, such as sexually transmitted diseases, are more likely to cause species extinction than diseases with density-dependent transmission (McCallum, 2008).

Immunology of DFTD

Allogeneic transfer of cells or tissues between unrelated individuals is normally prevented by histocompatibility barriers erected in all jawed vertebrates by a system involving the MHC. Two classes of MHC genes (class I and class II), both of which are highly genetically variable, function in adaptive immunity, cancer immunosurveillance and graft rejection (Janeway *et al.*, 2001). MHC class I is normally expressed by all nucleated cells, whereas MHC class II is normally expressed only in a subset of specialized antigen-presenting cells (Janeway *et al.*, 2001). Characterization of MHC expression and function is thus of central importance to understanding the immunology of clonally transmissible cancers.

Major histocompatibility complex class I and class II genes have extremely low levels of sequence divergence in the devil population from the Tasmanian east coast (Siddle *et al.*, 2007a, b). Siddle *et al.* (2007a) confirmed that MHC class I and II genes are expressed in DFTD tumors at the mRNA level, although further studies are required to determine whether MHC genes in DFTD are correctly translated, trafficked and displayed (Table 1). The lack of diversity at MHC loci, coupled with weak responses of east coast devils to allogeneic mixed lymphocyte culture, has led to the suggestion that this population may be functionally identical at MHC loci, thus permitting the spread of DFTD as an allograft (Figure 2; Woods *et al.*, 2007; Siddle *et al.*, 2007a; Kreiss *et al.*, 2008). However, as other marsupials have been reported to have weak responses to mixed lymphocyte culture, and antigens other than MHC also contribute to immunosurveillance, this model is yet to be confirmed (Montali *et al.*, 1998; Stone *et al.*, 1998; Janeway *et al.*, 2001).

Despite the allogeneic spread of DFTD within their population, devils have competent immune systems. Devil neutrophils are competent at bacterial phagocytosis and degradation, and lymphocyte proliferation can be stimulated by a variety of mitogens (Woods *et al.*, 2007; Siddle *et al.*, 2007a; Kreiss *et al.*, 2008). Lymphocyte proliferation responses varied greatly between individual Tasmanian devils, and lymphocytes from animals with DFTD responded similarly to those of healthy devils (Woods *et al.*, 2007; Kreiss *et al.*, 2008). These experiments confirm that DFTD does not induce general suppression of lymphocyte stimulation or

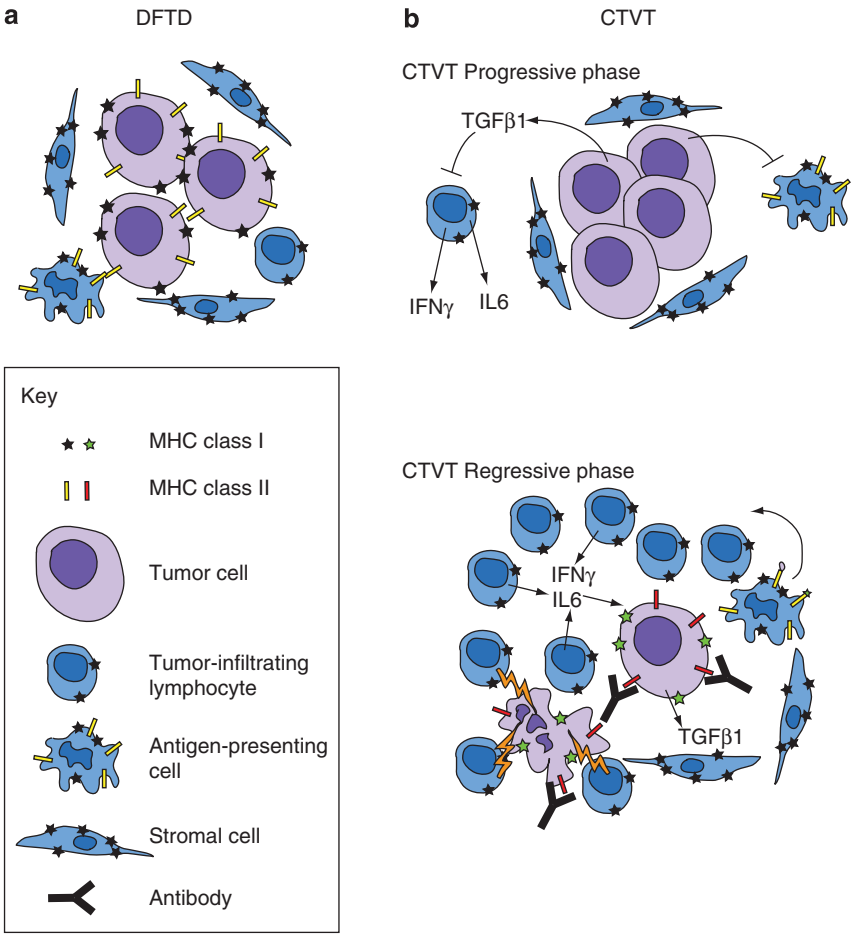

Figure 2 Models for devil facial tumor disease (DFTD) and canine transmissible venereal tumor (CTVT) immune evasion. (**a**) DFTD escapes immune detection because major histocompatibility complex (MHC) class I and class II lack functional diversity in Tasmanian devils. The tumor cells continue to proliferate as they are not recognized as foreign. (**b**) CTVT has distinct phases of growth, progressive and regressive. During the progressive phase, the tumor cells do not express MHC class I or class II, and the tumor secretes transforming growth factor-β1 (TGFβ1), a cytokine that inhibits tumor-infiltrating lymphocyte (including natural killer cell) cytotoxicity. Tumor cells may also inhibit some types of antigen-presenting cells. Tumor-infiltrating lymphocytes are present in low numbers. During the regressive phase, tumor-infiltrating lymphocytes increase in number, and their secretion of IFNγ and interleukin-6 (IL6) counteracts the repressive effects of tumor-derived TGFβ1, and induces MHC class I and class II expression in tumor cells. MHC expression reveals CTVT as an allograft, and it is rejected by both antibody-dependent and -independent cytotoxic processes. For details see text.

proliferation. Furthermore, devils are able to mount humoral responses to exogenous antigens and retain immunological memory (Woods *et al*., 2007; Kreiss *et al*., 2009). The discovery of antibodies recognizing DFTD in at least one devil (Obendorf and McGlashan, 2008), together with the observation of possible plasmacytosis in DFTD-affected devils (Woods *et al*., 2007), raises the possibility that at least some devils may mount humoral responses to DFTD.

There is currently no available vaccine, treatment or cure for DFTD, nor any evidence for resistance in the wild. Disease management options for the devil have been discussed extensively (McCallum and Jones, 2006; Jones *et al*., 2007; Lunney *et al*., 2008; McCallum, 2008). Insurance populations of captive devils or managed isolated wild-devil colonies are considered to be the most feasible option for maintaining the devil as a viable species. Controlling the indirect threat from feral species introduced to Tasmania such as the domestic cat and the European red fox will also be of crucial importance for protecting the devil in its natural habitat.

CTVT

Canine transmissible venereal tumor, also known as Sticker's sarcoma, is an infectious genital tumor that affects dogs (*Canis familiaris*; Figure 1). Spread by the physical transfer of cancer cells during coitus, CTVT is characterized by the appearance of lesions around the external genitalia that can affect both sexes of any breed of dog (Karlson and Mann, 1952; Brown *et al*., 1980; Cohen, 1985). The disease has a global distribution and has been documented in six continents (Cohen, 1985; Das and Das, 2000). CTVT and its transmissible phenotype were first documented in 1876 by Nowinsky (Nowinsky, 1876), and the tumor subsequently became a popular model for cancer biologists (Cohen, 1985; Das

and Das, 2000). However, despite 130 years of research into CTVT, many features of this unusual transmissible cancer remain poorly understood.

Canine transmissible venereal tumors first appear as small, firm, localized nodules, typically at the base of the glans penis in males, or in the vaginal vestibulum of females (Bellingham Smith and Washbourn, 1898; Rust, 1949; Kimeto and Mugera, 1974; Brown *et al*., 1980). Later-stage disease is characterized by pedunculated, exudative, ulcerating masses that can become as large as 10 cm or greater in diameter (Rust, 1949; Kimeto and Mugera, 1974; Brown *et al*., 1980; Mello Martins *et al*., 2005). CTVT usually remains localized, but there are numerous reports of CTVT metastasis, particularly to draining lymph nodes, cutaneous sites and visceral organs (Rust, 1949; Higgins, 1966; Kimeto and Mugera, 1974; Ndiritu *et al*., 1977; Brown *et al*., 1980; Dass and Sahay, 1989; Ferreira *et al*., 2000). One interesting feature of CTVT is that it sometimes undergoes spontaneous regression (see below).

Cytologically, CTVT cells appear as uniform round- to polyhedral-shaped centrally nucleated cells with a prominent nucleolus and characteristic cytoplasmic vacuoles. CTVT cells do not have many distinctive ultrastructural features (Murray *et al*., 1969; Hernandez-Jauregui *et al*., 1973; Cockrill and Beasley, 1975; Kennedy *et al*., 1977; Hill *et al*., 1984). Owing to its expression of lysozyme, α-1-antitrypsin, NSE and vimentin, CTVT has been proposed to be derived from the macrophage lineage (Table 1; Sandusky *et al*., 1987; Gimeno *et al*., 1995; Mozos *et al*., 1996; Marchal *et al*., 1997; Mukaratirwa *et al*., 2004). This diagnosis is supported by the observation that CTVT cells themselves may be parasitized by *Leishmania infantum*, an organism that normally infects macrophages (Albanese *et al*., 2002; Catone *et al*., 2003).

Clonal transmission was first suggested for CTVT due to the success of tumor transplant experiments such as those pioneered by Nowinsky (1876) and Sticker (1906). These experiments revealed that CTVT could be transmitted between unrelated dogs by the direct transfer of cancer cells or tumor tissue.

Cytogenetic studies also supported the clonal transmission theory. Whereas the constitutive chromosome number of dogs is 78, the karyotypes of CTVT tumors from Japan, Uganda, Jamaica, France, the United States, Nigeria and Russia all confirmed a chromosome number of 57–59 (Sofuni and Makino, 1963; Weber *et al*., 1965; Barski and Cornefert-Jensen, 1966; Kakpakova *et al*., 1968; Thorburn *et al*., 1968; Murray *et al*., 1969; Wright *et al*., 1970; Oshimura *et al*., 1973; Idowu, 1977; Richardson *et al*., 1987; Fujinaga *et al*., 1989). The CTVT karyotype included 15–17 metacentric or submetacentric chromosomes, in contrast to 2 in the normal dog karyotype. Array-based copy number analysis of CTVT also indicated significant deviation from normal, and strong correlations between tumors of diverse geographical origin (Rebbeck *et al*., 2009). The remarkable similarity between CTVT karyotypes from different continents, presumably from clones that have been geographically isolated for many years, suggests not only a common origin for globally distributed CTVT, but also highlights the fact that the clonal karyotype, despite its aneuploidy, is relatively stable.

Further evidence for clonal transmission of CTVT was provided by the identification of a LINE element insertion near the *MYC* locus in the CTVT genome (Table 1; Katzir *et al*., 1985). This genomic rearrangement has been identified in a large set of globally distributed CTVT tumors, but not in any other canine tissue, and is now considered diagnostic evidence for CTVT (Katzir *et al*., 1987; Amariglio *et al*., 1991; Choi *et al*., 1999; Chu *et al*., 2001b; Choi and Kim, 2002; Liao *et al*., 2003b; Murgia *et al*., 2006; Park *et al*., 2006; Vazquez-Mota *et al*., 2008; Rebbeck *et al*., 2009). It is possible that this rearrangement was present in the germ line of the CTVT founder, that it occurred somatically during the development of the founding CTVT tumor or that it occurred somatically in a CTVT clone that has subsequently achieved global distribution.

Clonal transmission for CTVT has also been confirmed by molecular genetic studies. Murgia *et al*. (2006) and Rebbeck *et al*. (2009) found that the pattern of microsatellite polymorphism in globally distributed CTVT tumors provided strong evidence for a monophyletic origin. Mitochondrial and MHC variants suggested that many modern CTVT clones belong to one of two distinct clades, each found in many regions around the world (Table 1; Murgia *et al*., 2006). Furthermore, variation between tumors was used to predict that the common ancestor for at least a subset of modern CTVTs existed between 47 and 470 years ago (Rebbeck *et al*., 2009) or between 250 and 2500 years ago (Murgia *et al*., 2006).

The genetic ancestry of CTVT has recently been investigated. By analysing variants in the *RPPH1* gene, Rebbeck *et al*. (2009) determined that CTVT probably arose from a dog or wolf, rather than from a more distantly related member of the canid family (Table 1). Furthermore, Murgia *et al*. (2006) used microsatellite polymorphism to perform a comparison of CTVT with 85 dog breeds and 8 wolves and found that CTVT clustered most strongly with wolves. MHC variants found in CTVT also indicated a strong phylogenetic relationship with wolves (Murgia *et al*., 2006). Assuming that CTVT arose in wolves, Rebbeck *et al*. (2009) used microsatellite divergence between CTVT and wolves to determine that the date of CTVT origin, as opposed to the date of the most recent common ancestor, was between 7800 and 78 000 years ago.

These studies indicate that CTVT was probably founded by a single wolf that existed between 7800 and 78 000 years ago. In recent times, a single clone has become dominant and has split into two clades, each with a broad global distribution. Thus CTVT is the oldest known somatic cell line.

CTVT life cycle

Canine transmissible venereal tumor is spread by coitus, and can be transferred either from male to female or

female to male (Bellingham Smith and Washbourn, 1898; Powell White, 1902). It can also be spread by licking, sniffing or scratching of affected areas; thus CTVT can sometimes develop extragenitally, either with or without genital involvement (Nowinsky, 1876; Feldman, 1929; Higgins, 1966; Ndiritu *et al*., 1977; Brown *et al*., 1980). CTVT transmission may be enhanced both by the extended period of canine sexual intercourse, which involves the mates being 'tied' due to the expansion of the penis within the female genital tract, and by the injuries to the genital mucosa that are frequently incurred as mates attempt to separate (Rust, 1949; Cohen, 1985).

Much has been learnt about the growth behavior of CTVT from experimental transplantation studies. Experimentally transferred CTVT tumors have three distinct phases of growth, described as progressive, stable and regressive (Wade, 1908; DeMonbreun and Goodpasture, 1934; Karlson and Mann, 1952; Epstein and Bennett, 1974; Hill *et al*., 1984; Cohen, 1985; Chu *et al*., 2001a). Tumors generally become palpable 10–20 days following experimental transfer (Karlson and Mann, 1952; Epstein and Bennett, 1974; Chu *et al*., 2001a). The initial progressive phase, which generally lasts for a few weeks, is characterized by a rapid increase in tumor volume with a doubling time of between 4 and 7 days, and an estimated loss of 50% of cells (Cohen and Steel, 1972; Epstein and Bennett, 1974; Chu *et al*., 2001a). During the subsequent stable phase, there is markedly slower tumor growth with a doubling time of approximately 20 days, and an estimated cell loss of 80–90% (Cohen and Steel, 1972). Following the stable phase, which can last from weeks to months to indefinitely, up to 80% of CTVT tumors enter a regressive phase during which the tumor shrinks and eventually disappears (Wade, 1908; Epstein and Bennett, 1974; Cohen, 1985; Chu *et al*., 2001a). The regressive phase generally lasts between 2 and 12 weeks, during which time tumors as large as $100\,cm^3$ can disappear completely (Wade, 1908; DeMonbreun and Goodpasture, 1934; Karlson and Mann, 1952; Epstein and Bennett, 1974; Cohen, 1985; Chu *et al*., 2001a). Alternatively, rather than entering the regressive phase, between 1 and 20% of transplanted tumors enter a second phase of rapid growth which progresses to metastasis (Wade, 1908; DeMonbreun and Goodpasture, 1934; Karlson and Mann, 1952; Epstein and Bennett, 1974).

The life history of naturally occurring CTVT is less well understood. Although an initial progressive phase and subsequent stable phase may be observed, spontaneous regression has not been well documented in naturally occurring CTVT (DeMonbreun and Goodpasture, 1934; Stubbs and Furth, 1934; Rust, 1949; Higgins, 1966; Brown *et al*., 1980; Perez *et al*., 1998; Mukaratirwa *et al*., 2004, 2006). In some regions, metastatic spread has been frequently observed in naturally occurring CTVT, and is thought to be particularly prevalent among dogs in poor general condition (Higgins, 1966). As CTVT is predominantly spread during coitus, canine reproductive biology may influence CTVT transmission. Male dogs, which are constantly sexually receptive, may have greater opportunity to spread CTVT, in contrast to females, which become sexually receptive only once every 6–7 months. Indeed Bellingham Smith and Washbourn (1898) observed that a single CTVT-affected male dog spread the disease to 11 of 12 females, and at least in some regions CTVT is naturally found with greater prevalence in females than in males (Karlson and Mann, 1952; Dass and Sahay, 1989; Scarpelli *et al*., 2008). If CTVT tumors in males become sufficiently large, they may obstruct preputial retraction (Wade, 1908; Mello Martins *et al*., 2005). As the penis is required to be unsheathed for coitus, it would be interesting to investigate whether such tumors have reduced transmission.

Immunology of CTVT

The course of CTVT is influenced by the immune system, with disease manifestation representing the outcome of tumor immune evasion strategies balanced against host immune responses. Experimental transfer of CTVT into immunocompromised individuals, such as neonatal puppies (Yang and Jones, 1973) or X-irradiated dogs (Cohen, 1973), results in continued progression of CTVT and widespread metastatic disease. Conversely, dogs that have recovered from CTVT have serum-transferable immunity to re-infection (Bellingham Smith and Washbourn, 1898; Crile and Beebe, 1908; Powers, 1968) and puppies born to mothers that have been exposed to CTVT are less susceptible to the disease (Yang and Jones, 1973). The transition from progressive to regressive phases of CTVT growth is accompanied by a marked increase in immune cell infiltration (Wade, 1908; Chandler and Yang, 1981; Hill *et al*., 1984; Trail and Yang, 1985; Perez *et al*., 1998; Barber and Yang, 1999; Gonzalez *et al*., 2000; Chu *et al*., 2001a; Hsiao *et al*., 2002; Mukaratirwa *et al*., 2004, 2006).

Major histocompatibility complex class I and class II are not expressed, or are expressed on only a very small subset of cells, during progressive CTVT growth, although there have been conflicting reports (Table 1; Epstein and Bennett, 1974; Cohen *et al*., 1984; Yang *et al*., 1987; Mizuno *et al*., 1994; Perez *et al*., 1998; Hsiao *et al*., 2002; Murgia *et al*., 2006). Interestingly, a markedly increased proportion of CTVT cells express MHC class I and class II in regressing tumors (Yang *et al*., 1987; Perez *et al*., 1998; Hsiao *et al*., 2002). Furthermore, CTVT cells can be induced to express MHC by exposure to supernatant of cultured regressive-phase CTVT cells and tumor-infiltrating lymphocytes, but not by progressive-phase CTVT cells and tumor-infiltrating lymphocytes (Hsiao *et al*., 2002).

The transition from progressive to regressive CTVT phase may be triggered by the induction of MHC expression caused by cytokine signaling by tumor-infiltrating lymphocytes (Figure 2). Hsiao *et al*. (2004) found that CTVT cells produce transforming growth factor-β1 (TGFβ1), and showed that this cytokine inhibited natural killer (NK) cell activity, as well as

tumor-infiltrating lymphocyte cytotoxity. The suppressive effects of TGFβ1 on NK cell-killing activity could be counteracted by the pro-inflammatory cytokine interleukin-6 (IL6), which is secreted by tumor-infiltrating lymphocytes (Hsiao *et al*., 2004). Hsaio *et al*. (2008) found that host-derived IFNγ acted synergistically with IL6 to induce MHC expression in CTVT cells. In addition, IL6 induced MHC expression in CTVT *in vitro* (Hsiao *et al*., 2008), and could induce MHC expression in combination with IL15 *in vivo* (Chou *et al*., 2009). A model has been proposed whereby progressive-phase CTVT avoids immune recognition by downregulating MHC class I and II and suppressing NK cell activity by the secretion of TGFβ1. Once a threshold level of IL6, secreted by tumor-infiltrating lymphocytes, is reached, the repressive effect of TGFβ1 on tumor-infiltrating lymphocyte IFNγ activity is released, allowing tumor-infiltrating lymphocytes to induce MHC class I and II expression in CTVT cells, triggering regression (Figure 2; Hsiao *et al*., 2004, 2008).

In addition to cell-mediated pathways, CTVT also triggers a humoral immune response. Antibodies recognizing CTVT antigens can be detected in the sera of CTVT-affected animals (McKenna and Prier, 1966; Cohen, 1972; Epstein and Bennett, 1974; Fenton and Yang, 1988). Although serum antibody levels did not correlate strongly with tumor volume, they were undetectable in the serum of puppies with metastatic CTVT, and few cells in CTVT metastases could be labeled with anti-CTVT antibodies (Epstein and Bennett, 1974; Fenton and Yang, 1988). Furthermore, Liao *et al*. (2003a) detected a CTVT-secreted factor that was specifically cytotoxic to B cells. Thus, although CTVT antibodies are not protective against established CTVT, they may slow tumor growth, protect against metastasis, participate in tumor cell cytotoxicity during the regressive phase and reduce susceptibility to subsequent CTVT infection (Powers, 1968; Cohen, 1980; Fenton and Yang, 1988).

Canine transmissible venereal tumor is antigenic in dogs and provokes both cell-mediated and humoral immune responses. The tumor is able to escape immune rejection by downregulating MHC, suppressing NK cells, killing B cells and preventing the maturation of dendritic cells (Hsiao *et al*., 2002, 2004; Liao *et al*., 2003a; Liu *et al*., 2008). However, CTVT may often eventually succumb to host defenses, and its final regression is accompanied by subsequent immunity. Although the triumph of the immune system over CTVT may reveal an inherent weakness in the tumor's defense strategy, it has also been suggested that natural regression may be an adaptation to maintain the viability of the host population (Murgia *et al*., 2006).

Discussion

Devil facial tumor disease and CTVT are the only two known naturally occurring clonally transmissible cancers. Comparison of these two cancers not only reveals common themes that have been favored during the evolution of the diseases, but also offers insight into the cancers' unique adaptations to their particular host species (Table 2). In addition, clonally transmissible cancers may have presented selective pressures that have not only influenced the evolution of devils and dogs, but may have more broadly shaped the evolution of allorecognition in multicellular eukaryotes.

Table 2 Comparison of DFTD and CTVT

	DFTD	*CTVT*
Host species	Tasmanian devil	Dog
Species of origin	Tasmanian devil	Wolf or dog
Distribution	Mainland Tasmania (excluding northwest)	Worldwide
Time of origin	15–20 years ago	7800–78 000 years ago
Body location	Face, oral cavity	External genitalia
Mode of transfer	Biting	Coitus
Histogenesis	Neuroendocrine	Myeloid
Metastasis	Common	Common in immune-compromised animals
Spontaneous regression	0%	Common in experimentally inoculated CTVT, prevalence in naturally occurring CTVT unknown
Mortality	100%, within 6–12 months after appearance of symptoms	Rare in experimentally inoculated CTVT, prevalence in naturally occurring untreated CTVT unknown
Treatment	None	Chemotherapy, radiation therapy
Effect on host population	Host population decline/possible imminent extinction	Probably little effect

Abbreviations: CTVT, canine transmissible venereal tumor; DFTD, devil facial tumor disease.

Both behavioral and genetic aspects of the biology of Tasmanian devils and of dogs may have favored the emergence of clonally transmissible cancers in these two species. The facial biting behavior of devils, and the extended and rough sexual intercourse of dogs offer routes for natural transmission for DFTD and CTVT. Both devils and dogs have experienced relatively recent population bottlenecks, caused by the island founder effect in the case of devils (~14 000 years ago) (Jones *et al*., 2004), and domestication (~15 000–100 000 years ago) followed by breed formation (~ <400–5000 years ago) in dogs (Vila *et al*., 1997; Savolainen *et al*., 2002; Parker *et al*., 2004). The low genetic diversity of the eastern Tasmanian population of devils, from which the DFTD founder was derived, may have enabled the establishment of DFTD (Jones *et al*., 2004; Siddle *et al*., 2007a). Furthermore, genetic analysis suggests that the animal that founded CTVT may have been homozygous at a number of loci (Murgia *et al*., 2006), and although there is no information about the genetic diversity of the population in which CTVT first arose, it is tempting to speculate that inbred populations may be more susceptible to the emergence of clonally transmissible cancer (Murgia *et al*., 2006; Siddle *et al*., 2007a; McCallum, 2008; Rebbeck *et al*., 2009).

Clonally transmissible cancers have the potential to promote adaptive evolution in their host populations. DFTD infection causes a significant reduction in individual fitness by causing death of affected devils early in their reproductive life. It seems likely that such conditions provide strong selection for life history traits such as precocial breeding, raising the possibility that natural selection could promote resilience in the devil population (Jones *et al*., 2008; Lachish *et al*., 2009).

Conversely, host biology and behavior may place evolutionary constraints on their cancers. Even as DFTD forces its host population toward extinction, transmission frequency has remained high, suggesting that there may be little pressure for this disease to reduce virulence (Lachish *et al*., 2007; McCallum, 2008). However, given that DFTD transmission may be enhanced by passing through the annual devil mating season, DFTD clones that kill their hosts less than a year after infection may have reduced fitness. In addition, it will be interesting to investigate whether clonally transmissible cancers are able to manipulate their hosts to enhance transmission, perhaps by enhancing host aggression (DFTD) or by stimulating sexual behaviors (CTVT) (Rebbeck *et al*., 2009). Indeed, de Brito *et al*. (2006) found differences in estrogen receptor expression in the vaginal epithelium between CTVT-affected and control female dogs during certain stages of the estrus cycle, suggesting the possibility of such a mechanism.

Parasitic cell lines may have been a driving force in the evolution of allo-recognition. Failure of self/non-self-recognition in colonial ascidians permits parasitic chimerism (Laird *et al*., 2005), a situation not dissimilar to clonally transmissible cancers. The MHC of jawed vertebrates is central to both adaptive immunity and allo-recognition, and clonally transmissible cancers may have provided selective pressure for the evolution of MHC diversity (Dingli and Nowak, 2006; Murgia *et al*., 2006; Kurbel *et al*., 2007).

The infectious dose of tumor cells required to transmit DFTD or CTVT is limited by the number of cells associated with a devil's penetrating canine tooth at the time of biting, or the number of cells sloughed from a dog's ulcerating genital lesion during mating. Thus, although the minimum number of cells required for cancer transmission has not been determined (most experimental CTVT transmission studies use 10^8 viable cells), it is likely that each new tumor is founded by a small number of cells, or even a single cell. Such transmission bottlenecks may provide strong selection for friability, a feature that is characteristic of both DFTD and CTVT (Brown *et al*., 1980; Thacher and Bradley, 1983; Jones *et al*., 2007). Tight transmission bottlenecks, particularly in asexual clones, also provide conditions for genetic drift and rapid fixation of neutral or deleterious alleles. Sequencing of transmissible cancer genomes will determine the extent of this effect.

The Tasmanian devil is restricted in both population size and habitat, and the rapid population decline caused by DFTD has led to the species being listed as endangered (Hawkins *et al*., 2009). Dogs, on the other hand, are a widespread species of least conservation concern, and little attention has been paid to the effect of CTVT on dog populations. However, the finding that CTVT can be experimentally transplanted into other *Canidae*, including coyotes (*Canis latrans*) (Cockrill and Beasley, 1979) and distantly related foxes (Sticker, 1906; Wade, 1908), raises the possibility that CTVT could become a conservation concern if it naturally entered endangered canid populations, such as that of the African wild dog (*Lycaon pictus*) (VonHoldt and Ostrander, 2006).

Although DFTD and CTVT are the only known clonally transmissible cancers that occur naturally, other clonally transmissible cancers have been derived in laboratory animal populations. A spontaneously arising sarcoma was found to be transmissible among a colony of Syrian hamsters (Brindley and Banfield, 1961; Cooper *et al*., 1964), and this cancer could even be experimentally transferred between individuals by mosquitoes (Banfield *et al*., 1965). A number of mouse cancer cell lines can be propagated by intraperitoneal injection through unrelated mouse strains (Carry *et al*., 1979; Hicks *et al*., 2006). Transmissible cancers with unknown etiology, some of which are transplantable, have been described in newts and fish (Champy and Champy, 1935; Lucke and Schlumberger, 1949). Thus although naturally occurring clonally transmissible cancer is probably rare, its incidence in wildlife may be underestimated.

Clonal cancer transmission can occur between humans by organ transplant, maternal–fetal transmission and fetal–fetal transfer of cancer cells (Kauffman *et al*., 2002; Tolar and Neglia, 2003; Sala-Torra *et al*., 2006). Furthermore, there has been a report in the medical literature of the transfer of a malignant sarcoma from a patient to his unrelated surgeon during an accidental injury during surgery (Gartner *et al*., 1996).

Devil facial tumor disease and CTVT are remarkable mammalian cell clones that have become asexual parasites and achieved widespread success in the colonization of their host species. As clonally transmissible cancers have arisen at least twice in natural populations in our time, it is likely that such entities have arisen multiple times over the course of evolutionary history, and that they have had significant impacts on the evolution of allo-recognition and the viability of species.

Conflict of interest

The author declares no conflict of interest.

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