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Illegitimate recombination between T cell receptor genes in humans and pigs (*Sus scrofa domestica*)

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Abstract T cell receptor (TCR) genes (*TRA*/*TRD*, *TRB* and *TRG*) reside in three regions on human chromosomes (14q11.2, 7q34 and 7p14, respectively) and pig chromosomes (7q15.3-q21, 18q11.3-q12 and 9q21-22, respectively). During the maturation of T cells, TCR genes are rearranged by site-specific recombination. Occasionally, interlocus recombination of different TCR genes takes place, resulting in chromosome rearrangements. It has been suggested that the absolute number of these "innocent" *trans*-rearrangements correlates with the risk of lymphoma. The aims of this work were to assess the frequencies of rearrangements with breakpoints in TCR genes in domestic pig lymphocytes and to compare these with the frequencies of corresponding rearrangements in human lymphocytes by using fluorescence in situ hybridization with chromosome painting probes. We show that frequencies of *trans*-rearrangements involving *TRA/TRD* locus in pigs are significantly higher than the frequency of translocations with breakpoints in *TRB* and *TRG* genes in pigs and the frequencies of corresponding *trans*-rearrangements involving *TRA/TRD* locus in humans. Complex structure of the pig *TRA/TRD* locus with high number of potential V(D)J rearrangements compared to the human locus may account for the observed differences. Furthermore, we demonstrated that *trans*-rearrangements involving pig *TRA/TRD* locus occur at lower frequencies in γδ T cells than in αβ T lymphocytes. The decrease of the frequencies in γδ T cells is probably caused by the absence of *TRA* recombination during maturation of this T cell lineage. High numbers of innocent *trans*-rearrangements in pigs may indicate a higher risk of T-cell lymphoma than in humans.

Keywords T cell receptor gene · γδ T lymphocytes · pig · chromosome rearrangements · interlocus recombination · T-cell lymphoma

Abbreviations

BAC	Bacterial artificial chromosome
FISH	Fluorescence in situ hybridization
PBL	Peripheral blood lymphocyte
PBMC	Peripheral blood mononuclear cells
PHA	Phytohaemagglutinin
PWM	Pokeweed mitogen
TRA	T cell receptor alpha locus
TRB	T cell receptor beta locus
TRD	T cell receptor delta locus
TRG	T cell receptor gamma locus
TCR	T cell receptor
VDJ	Variable, diversity and joining

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Introduction

T cells express either the αβ or γδ type of antigen-specific receptor. The structures of these receptors closely resemble those of immunoglobulins produced by B cells. There are four genes that encode T cell receptor (TCR) proteins: *TRA*, *TRB*, *TRD* and *TRG* (previous symbols TCRA, TCRB, TCRD and TCRG). *TRD* is located within *TRA*; therefore, these four TCR genes reside in three chromosome regions overall. *TRA*/*TRD*, *TRB* and *TRG* are located on 14q11.2, 7q34 and 7p14, respectively, in humans (HGNC, http://www.genenames.org) and on 7q15.3-q21, 18q11.3-q12 and 9q21-22, respectively, in pigs (Hiraiwa et al. 2001). During T cell maturation, TCR genes are rearranged by site-specific recombination between clusters of variable, diversity and joining (VDJ) gene segments.

T lymphocytes with αβ or γδ receptors arise from a common progenitor. *TRB*, *TRD* and *TRG*, coding for the β, δ and γ chains, respectively, are rearranged concurrently (Capone et al. 1998). Cells that productively rearrange both *TRG* and *TRD* shut down *TRB* rearrangement and express TCR γ and δ on the surface. Cells that productively rearrange *TRB* produce the β chain, which can pair with an invariant pre-T-cell receptor α. This pairing arrests further gene rearrangement and signals to the thymocyte to proliferate. Finally, the rearrangement of *TRA* starts, and the *TRD* segment located within *TRA* is deleted. When the rearrangement is successful, the T cell expresses the αβTCR on the surface and undergoes intrathymic selection for its ability to recognize self-MHC and self-peptide.

Occasionally, interlocus recombination of different TCR genes occurs, which results in the reciprocal translocation or inversion of chromosomes (Hecht et al. 1987; Kirsch 1994). Such illegitimate rearrangements occur at a low frequency in the peripheral blood lymphocytes of healthy individuals but occur at a markedly higher rate in patients with spontaneous chromosomal breakage syndromes such as ataxia telangiectasia (Kojis et al. 1991; Kobayashi et al. 1991) or Nijmegen breakage syndrome (Taalman et al. 1989). Hybrid antigen receptor genes might be functional and may contribute to the diversity of the immune response (Davodeau et al. 1994; Hinz et al. 2000). However, the process of DNA rearrangement also allows errors to occur; these errors include chromosomal aberrations resulting in the juxtaposition of oncogenes with TCR regulatory sequences, which are recurrent in several T cell malignancies (Boehm and Rabbitts 1989; Korsmeyer 1992; Marculescu et al. 2006). Indeed, the risk of cancer is increased in patients with chromosomal breakage syndromes (Hecht and Hecht 1990; Peterson et al. 1992).

To date, the frequencies of translocations caused by interlocus recombination of TCR genes have been determined in human peripheral blood lymphocytes using data from routine cytogenetic investigations carried out on human G-banded chromosomes (Hecht et al. 1987; Prieur et al. 1988; Tawn 1988). Furthermore, in humans and mice, these *trans*-rearrangements have been studied by PCR (Tycko et al. 1989; Lipkowitz et al. 1990; Kobayashi et al. 1991; Lista et al. 1997). Increased levels of *trans*-rearrangements were observed in farmers exposed to pesticides (Lipkowitz et al. 1992) and in oncological patients undergoing chemotherapy (Abdallah et al. 1995; Lopes et al. 2001). The effect of chemotherapy was transient, and the study in paediatric patients showed that higher levels were exhibited only in patients homozygous for the wild type of *CYP3A4* (Lopes et al. 2004). The results suggest that the absolute number of TCR *trans*-rearrangements may serve as a biomarker of genomic instability, genotoxic exposure and lymphoma risk (Lista et al. 1997; Kirsch 1997; Allam and Kabelitz 2006).

The proportions of αβ and γδ T lymphocytes in the circulation differ among species. Whereas less than 5 % of circulating T lymphocytes bear the γδTCR in humans and mice, γδ T cells may represent half of the peripheral blood lymphocyte (PBL) compartment in pigs (Yang and Parkhouse 1996) and other artiodactyls (Hein and Mackay 1991). To the best of our knowledge, TCR *trans*-rearrangements have not been studied in "γδ T cell high" species. The aims of this study were to assess the frequencies of reciprocal translocations with breakpoints in TCR loci in pig PBLs and to compare these with the frequencies of corresponding rearrangements in human lymphocytes. We used fluorescence in situ hybridization (FISH) and applied chromosome painting probes to analyse the chromosome rearrangements in both species. The study extends our knowledge of illegitimate V(D)J recombination.

Materials and methods

Animal and human blood donors

The experiments were performed using blood obtained from 10 young (5–12 months old) and 3 middle-aged

(5 years old) crossbred domestic pigs (*Sus scrofa domestica*) without any clinical symptoms of disease and from 6 healthy non-smoking men aged between 39 and 46 years.

PBL cultures

Phytohaemagglutinin (PHA)- and pokeweed mitogen (PWM)-stimulated whole blood lymphocyte cultures were prepared in RPMI1640 medium (Sigma-Aldrich, USA) supplemented with 20 % calf serum (Biotech, Prague, Czech Republic) and 2 % phytohaemagglutinin (Gibco, Grand Island, NY, USA) or 15 μg/ml pokeweed mitogen (Sigma). To prevent coagulation, heparin (Zentiva, Prague, Czech Republic) was added to pig lymphocyte cultures at a final concentration of 15 i.u./ml. The cultures were incubated at 37 °C for 72 h. One hour before the end of cultivation, colcemid (Sigma) was added at a final concentration of 1 μg/ml. The cells were collected by centrifugation, suspended in a hypotonic solution (0.075 M KCl) and fixed in 3:1 methanol/acetic acid according to the standard procedure. The cell suspensions were dropped on moistured microscopic slides.

Sorting and culturing of porcine T lymphocytes and γδ T lymphocytes

T lymphocytes ($CD3^+$) and γδ T lymphocytes ($\gamma\delta TCR^+$) were sorted using indirect magnetic labelling. First, peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation using Histopaque-1077 (Sigma-Aldrich). Isolated PBMCs were stained with anti-CD3 antibody (8E6, VMRD, WA, USA) or anti-γδTCR antibody (PGBL22A, VMRD). After the incubation and washing steps, goat anti-mouse IgG MicroBeads (Miltenyi Biotec) were added. Positive cells were sorted using a QuadroMACS separator (Miltenyi Biotec) according to the manufacturer's recommendations. The purity of the sorted cells was checked by flow cytometry using a BD LSRFortessa operated with BD FACSDiva™ Software (both Becton Dickinson, CA, USA). The purity was ≥99.4 % in all cases.

Sorted lymphocytes were cultured in complete RPMI1640 medium supplemented with serum, phytohaemagglutinin and heparin as described for PBL cultures. To induce the proliferation of sorted $\gamma\delta TCR^+$ lymphocytes, fresh medium was mixed 1:1 with medium from a 2-day whole blood lymphocyte culture. The cultures were incubated at 37 °C for 72 h in a 5 % CO_2 atmosphere.

Flow cytometry analysis of γδ T lymphocytes

Samples of whole blood lymphocytes or lymphocytes after stimulation were washed with a haemolytic solution (8.26 g NH_4Cl, 1 g $KHCO_3$ and 0.037 g EDTA per litre of distilled water) to remove the erythrocytes. After being washed with cell-wash solution (PBS containing 1.84 g/l EDTA, 1 g/l sodium azide and 4 ml/l gelatine), the leukocytes were stained with an anti-γδTCR antibody (PGBL22A, VMRD). Alexa Fluor® 488 goat anti-mouse IgG1 (Life Technologies, CA, USA) was used as the secondary antibody. Data were acquired and analysed on a BD LSRFortessa flow cytometer operated with BD FACSDiva™ Software.

Fluorescence in situ hybridization

Whole chromosome painting probes for pig chromosomes 7, 9 and 18 were prepared by laser microdissection according to the method described in a previous paper (Kubickova et al. 2002). Only good quality probes with high signal to noise ratio were used for the study. In young pigs no. 1–5 and middle-aged pigs no. 11–13, the first round FISH was carried out using probes for pig chromosomes 7 (Spectrum Green) and 9 (Spectrum Orange). To verify translocations involving chromosome 18, the slides were rehybridized with the probe for chromosome 18 (Spectrum Green) in combination with the probe for either chromosome 7 or 9 (Spectrum Orange). In young pigs no. 6–10, the first round FISH was carried out using probes for pig chromosomes 7 (Spectrum Orange) and 18 (Spectrum Green). In the second round FISH, the slides were rehybridized with the probe for chromosomes 9 (Spectrum Orange) and 18 (Spectrum Green). Hybridization was performed according to Musilova et al. (2007), with a slight modification. Briefly, 10 μl of the hybridization mixture (50 % formamide, 10 % dextran sulphate, 2× saline sodium citrate (SSC)) contained a minimum of 0.1 μg of each painting probe and 1–1.5 μg of pig competitor DNA (Applied Genetics Laboratories, Melbourne, FL, USA). After overnight hybridization, the slides were washed in 0.4× SSC/0.3 % igepal (pH 7.0) at 72 °C for 2 min.

The break apart probe for porcine *TRA/TRD* locus consisted of two differently labelled BAC probes flanking the gene. BAC constructs were obtained from

the Children's Hospital of Oakland Research Institute (CHORI): CH242-436B1 for proximal and CH242-105M6 for distal region with respect to the centromere. Clones were labelled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche Diagnostics GmbH, Mannheim, Germany) using a nick translation kit (Abbott, Des Plaines, IL, USA) and used in subsequent FISH. Biotin- and digoxigenin-labelled BAC probes were detected by fluorescein avidin (Vector Laboratories, Burlingame, CA, USA) and anti-digoxigenin-rhodamine (Roche), respectively.

Commercially available whole chromosome painting probes (Cambio, Cambridge, UK) for chromosomes 7 (FITC) and 14 (biotin labelled; detected by avidin-Cy3) and arm-specific painting probes (MetaSystems, Altlussheim, Germany) for chromosomes 7p (fluorescein) and 7q (Texas Red) were used for FISH experiments in the human samples according to the manufacturer's instructions.

Analysis

Slides were examined under an Olympus fluorescence microscope or under a Zeiss microscope equipped with the slide scanning system Metafer (MetaSystems). ISIS (MetaSystems) software was used for image analysis. A minimum of 5,000 metaphases from each culture were investigated. Slides prepared from different pig lymphocyte subsets were coded for a blind analysis. Only cells with all painted chromosomes present were classified. The findings were checked by two evaluators.

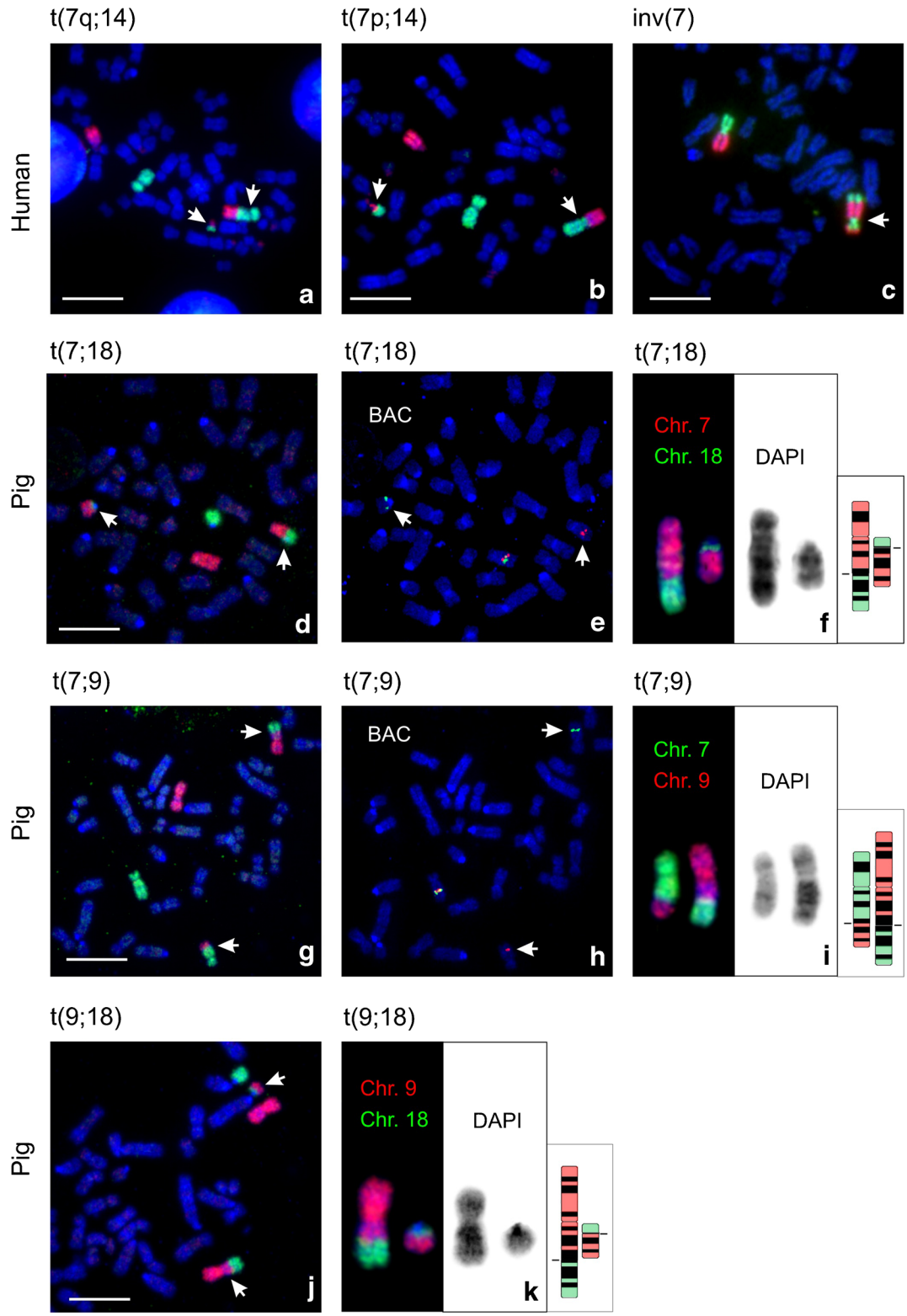
The frequencies of respective chromosome rearrangements in pigs and humans were analysed using a chi-squared test. Differences between groups were considered significant if probability values of $P<0.05$ were obtained.

Results

Chromosome rearrangements with breakpoints in TCR genes were studied using painting probes hybridized to metaphase lymphocytes (Fig. 1). The mean frequencies of t(7q;14), t(7p;14) and inv(7) in PHA-stimulated PBLs from the six men were 5.6×10^{-4}, 4.3×10^{-4} and 4.3×10^{-4}, respectively (Fig. 2, Supplementary Table 1). Rearrangements between *TRB* and *TRG* include besides inversions also one translocation t(7p;7q) between homologous chromosomes found in man no. 1. There was no significant difference in the frequency of any of these three rearrangements.

The frequencies of *trans*-rearrangements in domestic pigs were determined in PHA-stimulated whole blood lymphocytes of 10 young and 3 middle-aged animals (Fig. 2, Supplementary Table 2). Differences between two groups of young animals examined by different combinations of painting probes (see Materials and methods) were not significant. As regards the age, we did not observe significant differences in numbers of *trans*-rearrangements between young and middle-aged pigs, although the frequency of t(7;18) was slightly lower in middle-aged animals ($P=0.058$). To assess the baseline frequencies of chromosomal translocations with breakpoints in T cell receptor genes in pigs, we combined the data obtained from all 13 animals. The total average frequencies of t(7;18), t(7;9) and t(9;18) in porcine PHA-stimulated peripheral blood lymphocytes were 22.8×10^{-4}, 18.1×10^{-4} and 1.5×10^{-4}, respectively. The frequencies of translocations involving chromosome 7 (with breakpoints in *TRA*/*TRD*) were significantly higher ($P<0.001$) than the frequency of t(9;18) (with breakpoints in *TRB* and *TRG*). Comparison of the findings between pigs and humans showed significantly ($P<0.001$) higher frequencies of both translocations involving the *TRA*/*TRD* gene and the total frequency of

Fig. 1 Chromosome rearrangements with breakpoints in TCR genes. Human metaphase cells with **a** translocation t(7q;14), as determined by FISH with probes for chromosomes 7 (*green*) and 14 (*red*); **b** t(7p;14), as determined by FISH with probes for chromosomes 7 (*green*) and 14 (*red*); and **c** inversion inv(7), as determined by FISH with painting probes for chromosome arms 7p (*green*) and 7q (*red*). Translocation t(7;18) in pigs: **d** a metaphase cell after FISH with painting probes for chromosomes 7 (*red*) and 18 (*green*); **e** the same metaphase cell rehybridized with BAC probes: CH242-436B1 for proximal (*red*) and CH242-105M6 for distal (*green*) region flanking the *TRA/TRD* gene; and **f** translocated chromosomes after painting, counterstained with DAPI (converted to black and white and inverted), and their diagram. Translocation t(7;9) in pigs: **g** a metaphase cell after FISH with painting probes for chromosomes 7 (*green*) and 9 (*red*); **h** the same metaphase cell rehybridized with BAC probes: CH242-436B1 for proximal (*red*) and CH242-105M6 for distal (*green*) region flanking the *TRA/TRD* gene; and **i** translocated chromosomes after painting, counterstained with DAPI (converted to black and white and inverted), and their diagram. Translocation t(9;18) in pigs: **j** a metaphase cell after FISH with painting probes for chromosomes 9 (*red*) and 18 (*green*); and **k** translocated chromosomes after painting, counterstained with DAPI (converted to black and white and inverted), and their diagram. *Arrows* show translocated chromosomes in metaphase cells. *Scale bar* 10 μm


t(7q;14)
t(7p;14)
inv(7)
Human
a
b
c
t(7;18)
t(7;18)
t(7;18)
Pig
BAC
Chr. 7
Chr. 18
DAPI
d
e
f
t(7;9)
t(7;9)
t(7;9)
Pig
BAC
Chr. 7
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t(9;18)
t(9;18)
Pig
Chr. 9
Chr. 18
DAPI
j
k

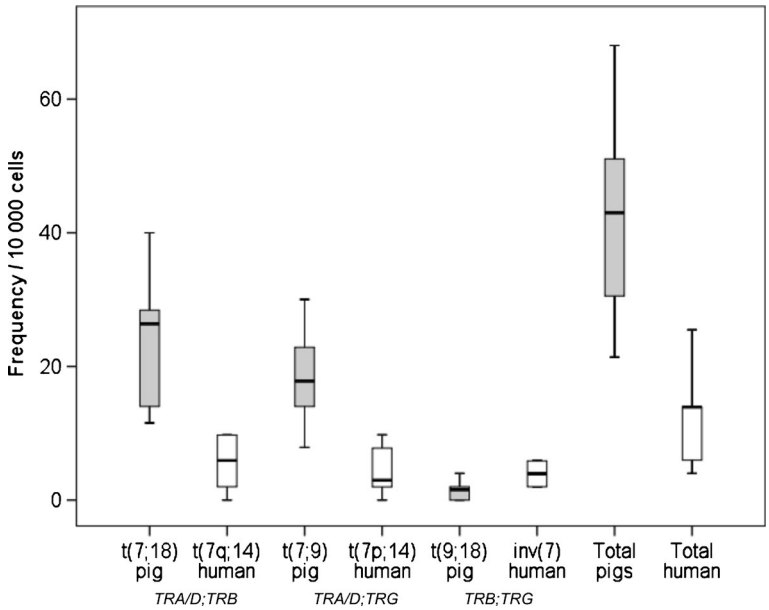

Fig. 2 Box plot showing the frequencies of *trans*-rearrangements in pigs and humans: the *vertical height* of each box represents the 25–75 % data range; the *horizontal line* within each box represents the median value; and the *upper and lower extensions* represent the maximum and minimum values. Data are representative of investigation of 71,814 metaphase cells in 13 pigs and 30,377 metaphase cells in 6 humans

all translocations in pigs, although the frequency of translocations with breakpoints in *TRB* and *TRG* was higher in humans (P=0.017). The distributions of the frequencies of the respective translocations in pigs and humans are shown in Fig. 2.

Pigs are among the "γδ T cell high" species. The percentage of γδ T cells in PBLs and in lymphoblasts after stimulation was measured in five young pigs and two middle-aged animals (Table 1). The proportion of γδ T cells was higher in younger pigs. To test the contribution of γδ T cells to the frequencies of *trans*-rearrangements, we investigated PHA-stimulated cultures of separated γδ T lymphocytes in five young domestic pigs and in cultures of total separated T lymphocytes in four of these animals (Fig. 3, Supplementary Table 3). Significantly fewer t(7;18) and t(7;9) translocations were observed in the separated γδ T lymphocytes than in either the separated T lymphocyte culture (P<0.001 and P=0.002, respectively) or the PBL culture (P<0.001 for both translocations). There was no significant difference between the findings for PBLs and those for T lymphocytes. These data indicate that *trans*-rearrangements involving *TRA/TRD* locus occur at lower frequencies in γδ T cells than in αβ T lymphocytes. Despite this decrease, the frequencies of t(7;18) and t(7;9) in γδ T cells were still significantly higher (P=0.006 and P=0.002, respectively) than the frequency of t(9;18).

To assess the effect of mitogen stimulation on the frequency of *trans*-rearrangements, we examined PWM-stimulated whole blood cultures and compared the results with those of PHA-stimulated cultures in the group of three middle-aged animals (Supplementary Table 4). No significant differences were observed between the groups as regards particular translocations. Only when pooled, the total number of *trans*-rearrangements was higher (P=0.042) in PWM-stimulated cultures than those in PHA-stimulated cultures. It is necessary to perform more experiments in order to ascertain whether stimulation with different mitogens has an effect on the frequency of *trans*-rearrangements.

Table 1 Percentage of γδ T cells in pig peripheral blood lymphocytes and in lymphoblasts in 3-day cultures

Pig no.	δ T cells (%) in PBL	Mitogen	γδ T cells (%) in lymphoblasts
1	47.5	PHA	27.8
2	40.6	PHA	17.4
3	56.1	PHA	25.7
4	33.1	PHA	10.9
5	34.4	PHA	18.4
6	11.5	PHA	2.5
		PWM	5.7
7	12.6	PHA	2.4
		PWM	3.9

Pig no. 1–5, young animals; pig no. 6 and 7, middle-aged animals

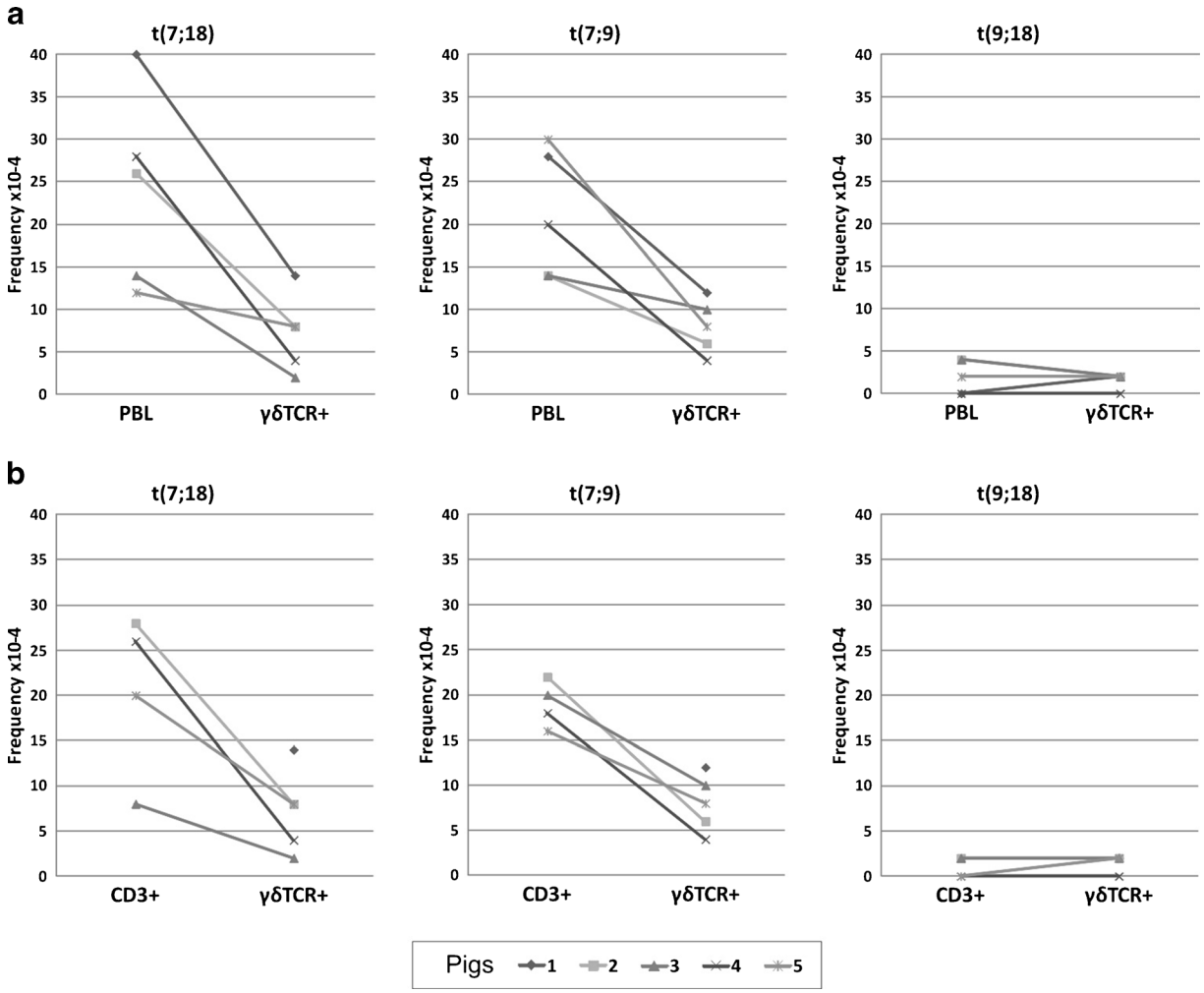

Fig. 3 Frequencies of respective *trans*-rearrangements in individual pigs in whole blood lymphocytes and γδ T lymphocytes (**a**) and in T lymphocytes and γδ T lymphocytes (**b**). In each animal, 5,000 metaphases were investigated per culture

Chromosome 7 was most frequently involved in translocations detected by painting probes in pigs. To verify the breakpoints in this chromosome, differently labelled BAC probes flanking *TRA/TRD* locus were used for subsequent FISH in some experiments. Only one out of 52 investigated translocations t(7;18) and none out of 45 t(7;9) exhibited a breakpoint in a locus different from *TRA/TRD*.

Rearrangements with chromosomes other than those carrying TCR genes were rarely detected during examination. In PBL cultures of young pigs no. 1–5, we observed six such translocations involving chromosome 7 (2.4/10,000 cells) and three translocations involving chromosome 9 (0.1/10,000 cells). Using BAC probes, we confirmed breakpoints in *TRA/TRD* locus in all six translocations involving chromosome 7. As regards rearrangements involving chromosome 9, painting results indicate that the breakpoints are in loci different from *TRG* in two out of three observed translocations.

Discussion

In this paper, we report the frequencies of translocations with breakpoints in TCR loci in porcine PBLs, separated T lymphocytes and separated γδ T lymphocytes using FISH with whole chromosome painting probes, and we compare these frequencies with the frequencies of the corresponding *trans*-rearrangements in human peripheral blood lymphocytes. The number of illegitimate recombinations between TCR genes in pigs was assessed for the first time.

We discovered that frequencies of *trans*-rearrangements involving *TRA/TRD* locus in pigs are higher than the frequency of translocations with breakpoints in *TRB* and *TRG* genes and the frequencies of corresponding *trans*-rearrangements involving *TRA/TRD* locus in humans (Fig. 2). It has been shown that the frequency of chromosome breaks and translocations involving the TCR genes correlates with the potential number of V(D)J rearrangements in the gene (Huang et al. 2007). This can explain the differences in frequencies of rearrangements between humans and pigs reported in this study. The porcine *TRA*/*TRD* has a more complex structure than the human and murine *TRA*/*TRD* (Uenishi et al. 2009). An increase in the number of *TRDV* and *TRDD* segments, which provide combinatorial diversity and explain the broad diversity of δ chain molecules in pigs, may also increase the probability of illegitimate *trans*-rearrangement between *TRA*/*TRD* and other TCR loci. Porcine *TRB* harbours three functional D-J-C cluster units (Eguchi-Ogawa et al. 2009); in contrast, human and mouse *TRB* has only two. An extensive *TRD* gene repertoire and duplication within the *TRB* locus have also been reported in other "γδ T cell high" species such as cattle (Conrad et al. 2002; Connelley et al. 2009; Herzig et al. 2010) and sheep (Antonacci et al. 2005, 2008). Studies of frequencies of translocations with breakpoints in TCR genes using whole chromosome painting probes in mice were performed on only a limited number of metaphases (Huang et al. 2007; Bowen et al. 2013). However, no rearrangement found among more than 1,000 evaluated cells suggests that frequencies in mice are probably lower than those in pigs and are rather similar to the frequencies in humans. To the best of our knowledge, *trans*-rearrangements have not been studied in cattle or sheep.

Spatial proximity of double strand breaks is thought to be a major factor contributing to recurrent chromosome translocations. Bowen et al. (2013) did not observe significant differences between inter-TCR distances and distances between TCR loci and non-antigen receptor locus in thymocytes undergoing V(D)J recombination in wild type and ataxia telangiectasia mutated (ATM) deficient mice. However, accessibility of TCR genes for interlocus recombination in pigs and humans can be influenced by the chromosome rearrangements which occurred during karyotype evolution after the diversification of these species. These rearrangements located TCR genes on two separate chromosomes in humans but three chromosomes in pigs, in synteny with different chromosomal elements (Goureau et al. 1996).

As pigs are among the "γδ T cell high" species, we also investigated the contribution of γδ T cells to the frequencies of *trans*-rearrangements. Our results suggest that mainly αβ T lymphocytes account for the high incidence of illegitimate recombination involving *TRA*/*TRD* in porcine lymphocytes. *TRB*, *TRD* and *TRG* genes are rearranged concurrently during maturation of both αβ and γδ T lymphocytes, whereas the *TRA* gene is rearranged only in the αβ lineage. The long accessibility of the *TRA*/*TRD* locus for recombination during the maturation of αβ T lymphocytes could account for the higher frequencies of translocations involving this locus in pig lymphocytes bearing the αβTCR than in those bearing the γδTCR. *Trans*-rearrangements in γδ T lymphocytes have not been studied in humans.

In the previous studies carried out on human G-banded chromosomes, the frequencies of chromosome rearrangements with breakpoints in TCR loci were reported to be 3.1×10^{-4} to 4.9×10^{-4} for t(7q;14), 2.6×10^{-4} to 6.2×10^{-4} for t(7p;14) and 0.2×10^{-4} to 3.7×10^{-4} for inv(7) (Hecht et al. 1987; Prieur et al. 1988; Tawn 1988). The frequencies of both translocations t(7;14) do not significantly differ from the results in our study. As regards to inv(7), our result is in agreement with those by Prieur et al. (1988) and Tawn (1988), but is significantly higher ($P<0.001$) than the frequency reported by Hecht et al. (1987). Apart from the method used, the studies differed in the strategy. Hecht et al. (1987) investigated a large number of individuals (2,595) but only a limited number of metaphase cells (in most cases 20) per person. On the contrary, we investigated more than 5,000 cells per person from 6 individuals.

Prieur et al. (1988) reported that the frequencies of translocations or inversions with breakpoints in TCR genes are higher in newborns than in adults. However, they examined only approximately 9,000 metaphases from eight individuals, and their results were not significant. Other studies based on PCR detection of recombination between γ variable and β diversity-joining sequences that corresponds to inv(7) in humans have reported a slight increase in frequency from birth to adulthood and then a decrease with increasing age (Ballinger et al. 2002; Meydan et al. 1999). In our study, we did not find any significant difference between the groups of young and middle-aged pigs. The frequency of t(7;18) was slightly higher in young pigs than in middle-aged animals (Supplementary Table 2), although

the former had a higher proportion of γδ T cells which exhibit fewer trans-rearrangements than αβ T cells (Table 1). However, the number of investigated animals was not sufficient to draw conclusions about the effect of age on the frequency of rearrangements involving TCR genes.

The process of DNA rearrangements during maturation predisposes lymphoid cells to aberrant DNA rearrangements involving the fusion of TCR loci to loci unrelated to the antigen receptor genes, which may be oncogenes. Such fusion to an oncogene may result in lymphoid malignancy. Rearrangements at the *TRD* locus are particularly common in lymphoma patients with juxtaposition of oncogene and TCR loci (Larmonie et al. 2013). It has been suggested that the absolute number of "innocent" *trans*-rearrangements with both breakpoints in TCR gene loci correlates with the risk of lymphoma, regardless of whether that risk is based on an inherited predisposition or acquired exposure. An assay of *trans*-rearrangements may serve as a biomarker of lymphoma risk (Lipkowitz et al. 1992; Lista et al. 1997; Kirsch 1997; Lopes et al. 2001; Allam and Kabelitz 2006). An increased number of innocent *trans*-rearrangements involving *TRA*/*TRD* in pigs may thus indicate a higher risk of T-cell lymphoma than in humans. In fact, malignant lymphoma is the most common tumour disease in pigs, and it is primarily observed in young animals (Misdorp 2003). The rates have been estimated to be 2–6.5 animals per 100,000 slaughtered swine (Jacobs et al. 2002). Even though B-cell lymphomas can be assumed to predominate in pigs as they do in humans, T and T-cell-rich B-cell lymphomas were described in pigs (Tanimoto and Ohtsuki 1998; Ogihara et al. 2012). However, their prevalence is unknown. As thousands of pigs slaughtered in abattoirs undergo veterinary meat inspection, a sufficient number of samples is available despite a low prevalence of T lymphomas. Thus, the pig can serve as a model for naturally occurring lymphomas of this type and provide evidence of a relationship between the frequency of innocent *trans*-rearrangements involving *TRA*/*TRD* and the occurrence of T-cell lymphomas. The pig is now the most common large laboratory animal species. Owing to the physiological, morphological and genomic similarities between pigs and humans, pigs became important biomedical models for cancer and other human diseases. Their large size makes them ideal models for preclinical studies of drugs as well as imaging, chemotherapy and radiation, which cannot be accurately studied in small animal models (Kuzmuk and Schook 2011).

In conclusion, we assessed the frequencies of chromosome rearrangements that occurred via illegitimate recombination between TCR loci in pig and human lymphocytes, by using FISH with painting probes. The frequencies of both translocations involving pig *TRA*/*TRD* were significantly higher than the frequency of translocations with breakpoints in the *TRB* and *TRG* genes in pigs and the frequencies of corresponding *trans*-rearrangements involving *TRA*/*TRD* in humans. Complex structure of the pig *TRA/TRD* locus with high number of potential V(D)J rearrangements compared to the human locus may account for the observed differences. *Trans*-rearrangements involving *TRA*/*TRD* were significantly lower in γδ T cells than in αβ T lymphocytes in pigs. The decrease of the frequencies in γδ T cells is probably caused by the absence of *TRA* recombination during maturation of this T cell lineage.

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Conflict of interest Petra Musilova, Jitka Drbalova, Svatava Kubickova, Halina Cernohorska, Hana Stepanova and Jiri Rubes declare that they have no conflict of interest.

Ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. All institutional and national guidelines for the care and use of laboratory animals were followed.

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