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A large proportion of bovine T cells express the γδ T cell receptor and show a distinct tissue distribution and surface phenotype

Charles R. Mackay and **Wayne R. Hein**

Basel Institute for Immunology, Grenzacherstrasse 487, CH-4058 Basel, Switzerland

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Abstract

The numbers, phenotype, and tissue distribution of γδ T cells in cattle were studied using two monoclonal antibodies (mAbs) which react with the bovine γδ T cell receptor (TCR). Both mAbs stained 20–40% of T cells in peripheral blood, and immunoprecipitated molecules of 44 and 36 kd (reduced) and 70–80 kd (non-reduced). In cattle the majority of circulating γδ T cells showed a distinct surface phenotype; they expressed T19, a 215 kd molecule described in sheep and cattle which marks only γδ T cells. Bovine γδ T cells were also CD2⁻, CD4⁻, and mostly CD8⁻, and failed to express CD6, a molecule possibly involved in T cell activation. The distribution of γδ T cells in cattle lymphoid tissues differed markedly from that in humans, in that bovine γδ T cells were concentrated around lymph node trabeculae and were usually sparse or absent from the B cell and T cell domains of lymph nodes. Like most other species studied, γδ T cells in cattle were localized to epithelial surfaces, particularly within the skin and intestine, indicating that it was at these sites where γδ T cells functioned. Our results provide further evidence for the unusual localization, recirculation pattern, and phenotype of γδ T cells, and also show that some features of γδ T cells can differ quite markedly from species to species.

T cells bearing the γδ T cell receptor (TCR) were identified only 3 years ago (1–6), and their function is still uncertain. However, one distinguishing feature of γδ T cells is their specific homing and localization to certain epithelial surfaces (7–12), which suggests that they play a role in the elimination of infected or transformed epithelial cells. Unlike the αβ T cell subset, at least a proportion of γδ T cells recognize antigen in a major histocompatibility complex (MHC)-unrestricted fashion (13,14), or in the context of non-classical class I antigens such as Qa-1 (15). Moreover, γδ T cells usually lack CD4 or CD8, two accessory molecules employed by αβ T cells for the recognition of antigen plus MHC

The relative contributions of γδ versus αβ T cells towards immune defense may differ significantly between species since the proportions of these two T cell types can vary quite widely (12,16,17). We have characterized γδ T cells in animals in one evolutionary niche, the ruminants, and have examined which properties of γδ T cells are conserved through evolution. Here we show that cattle, like sheep, have very large numbers of γδ T cells, and that these cells differ in many ways from γδ T cells of other species.

To characterize the γδ TCR and γδ T cells in cattle, we took advantage of the cross-reactivity of anti-γδ monoclonal antibodies (mAbs) which were originally raised against the γδ TCR of sheep (12). A FACS analysis of bovine peripheral blood lymphocytes (PBL) with several mAbs is shown on the right side of Fig. 1. The mAb 86D (anti-γδ) reacted with a large proportion of bovine PBL (18% in this experiment), and another γδ mAb, 69C, showed a similar fluorescence profile (not shown). Both mAbs immunoprecipitated from surface-labeled PBL the bovine γδ TCR (Fig. 1) which migrated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) with a molecular size of 44 and 36 kd (reduced) and 70–80 kd (non-reduced). In addition to the 44 and 36 kd bands, bands of 55 and 32 kd were also evident when an anti-C_δ serum was used (lane 3, arrows). This antiserum was raised against a peptide corresponding to a highly

Correspondence to: C. R. Mackay, as above

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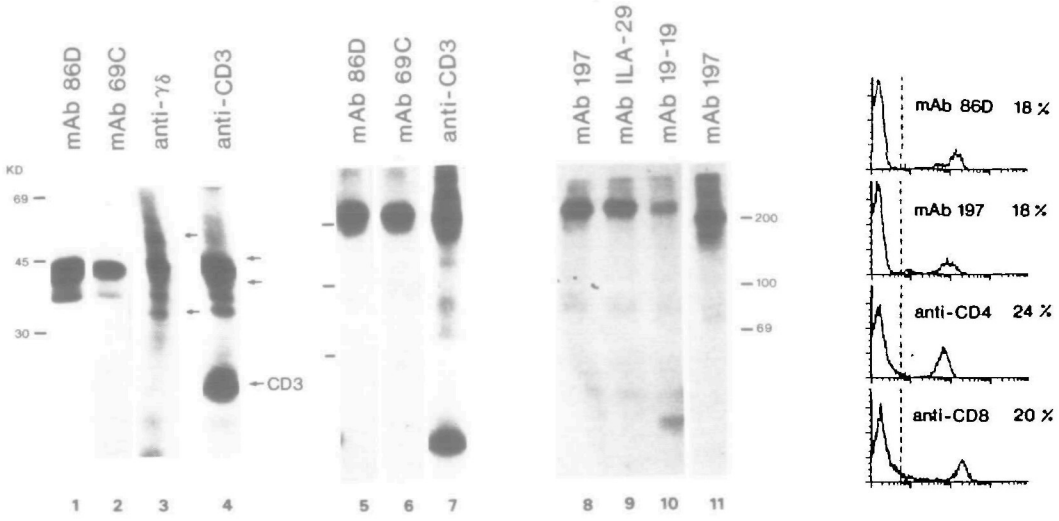

Fig. 1. SDS – PAGE analysis of the γδ TCR and the T19 molecule immunoprecipitated from surface-labeled bovine PBL. The lymphocyte preparation used to prepare the lysate contained 24% CD4, 20% CD8, and 18% γδ T cells, as assessed by immunofluorescence staining (see right hand side) Lanes 1 – 4 show material immunoprecipitated by mAbs 86D and 69C (both anti-γδ), an anti-δ serum (11), and an anti-CD3 serum (see ref 19) All four lanes were run under reducing conditions The anti-δ serum (lane 3) immunoprecipitated two bands (arrowed) which were absent from lanes 1 or 2. In lane 4 two bands were present (arrowed) which were absent from lane 3, and presumably represent αβ TCR bands. Lanes 5 – 7 were run under non-reducing conditions. Lanes 8 – 10 show material immunoprecipitated by three independently derived mAbs, all of which recognize the 215 – 220 kd T19 molecule Under non-reducing conditions, the T19 molecule migrated slightly faster (lane 11) The procedure for immunoprecipitation and SDS – PAGE followed previously described methods (4), except that in the present experiments the lysis buffer consisted of 10 mM Tris, pH 8 0, 10 mM CHAPS (BioRad), 10 mM iodoacetamide, and 2 mM PMSF Procedures for immunofluorescence staining and flow cytometry were as previously described (4)

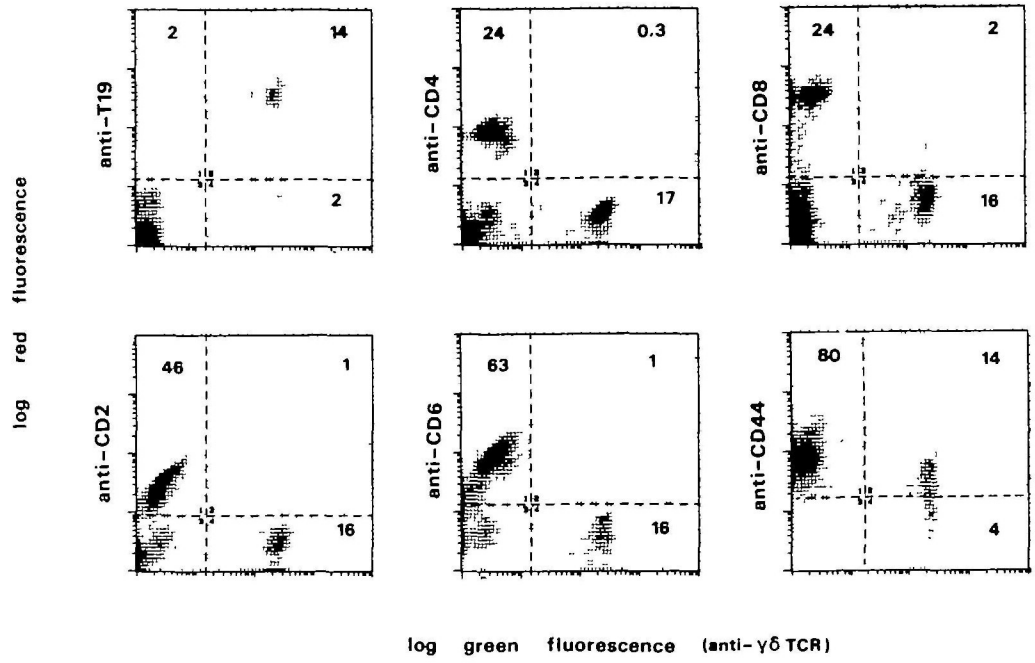

Fig. 2. Two-color immunofluorescence analysis of cattle PBL with the anti-γδ mAb 86D and various other markers. Green fluorescence in all plots represents staining with FITC-labeled anti-γδ mAb 86D Red fluorescence represents staining with biotinylated mAb (as indicated) followed by avidin-PE. Quadrants were constructed in each plot according to control antibody staining, and the percentage of cells stained red only, dual stained, or stained green only is indicated in each quadrant. Sixty per cent Percoll was used for preparation of mononuclear cells from cattle blood. Immunofluorescence staining of single cell suspensions was as described (4), except that a FACScan flow cytometer was used (Becton Dickinson, Sunnyvale, CA) Lymphocytes were gated according to their forward and side scatter. In this preparation of PBL, γδ T cells identified by mAb 86D comprised ~17% of cells. Other mAbs used were 197 (anti-T19) (19), ILA-11 (anti-CD4) (25), 38-65 (anti-CD8) (4), CH128A (anti-CD2) (26), ILA-27 (anti-CD6) (22), and 25-32 (anti-CD44) (27).

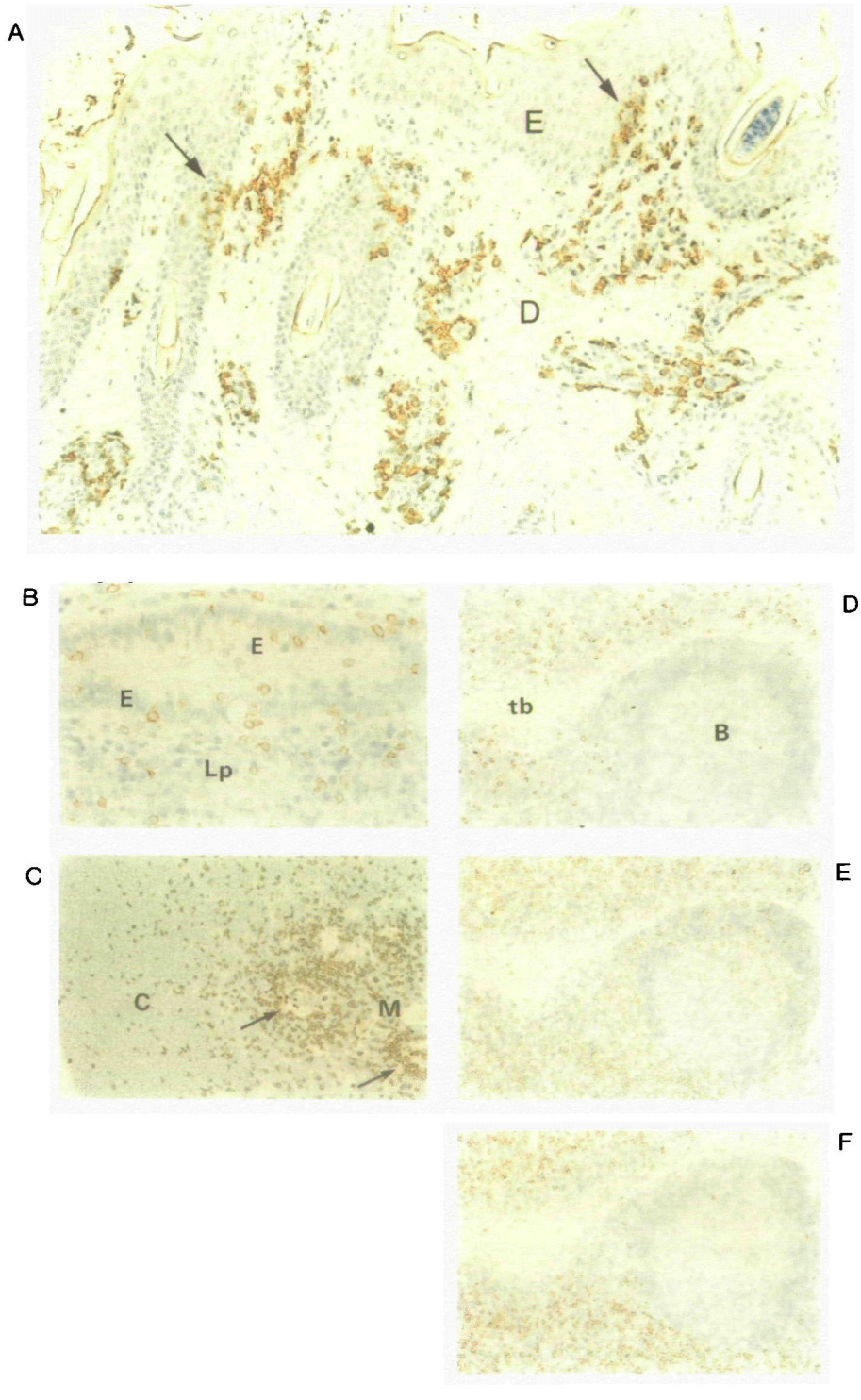

Fig. 3. Distribution of γδ T cells within bovine tissues. Frozen sections were prepared from snap-frozen tissue, and were stained with mAb 86D using the immunoperoxidase technique, as described (4). (a) Skin. The majority of 86D+ cells were of an irregular shape, and localized to the dermis (D). Small numbers of stained cells were also situated within the epidermis (E) and usually occurred as clusters (arrowed). Magnification ×240. (b) Intestinal epithelium, showing 86D+ cells situated within the lamina propria (LP) and within the epithelium (E). ×150. (c) Thymus tissue section stained with mAb 86D, showing positively stained cells localized mainly within the medulla (M) and also scattered throughout the cortex (C). In the medulla an accumulation of stained cells can be seen surrounding Hasall's corpuscles (arrowed). ×100. (d–f) Serial lymph node tissue sections stained with mAb 86D, anti-CD4, and anti-CD8, respectively. B = B cell follicle; T = T cell area; tb = trabecula. ×150.

Table 1. Reactivity of mAbs with bovine thymocytes, PBL, and lymph node cells

	% of cells stained by				
	mAb 86D	mAb 69C	anti-T19	anti-CD4	anti-CD8
Thymocytes	1 5	1 4	0.5	88	86
PBL	18	18	18	24	20
Lymph node cells	1 6	1 3	1.2	50	25

Figures are from a single, representative animal, ~8 months of age Lymph node cells were from the parathymic lymph node. Cells were reacted with mAb, followed by goat anti-mouse Ig-FITC and were analyzed on a FACScan flow cytometer At least five animals, 8 – 12 months of age, were examined, and in all cases the staining values were similar to those reported here for this one animal

conserved region of mammalian C_δ [amino acids 159 – 187 of human C_δ (11)]. When an anti-CD3 serum was used to isolate TCR material, the 55, 44, 36, and 32 kd bands (all presumably γδ TCR) were present, as were other bands which presumably represent αβ TCR (lane 4, arrowed). The presence of the 55 and 32 kd bands in the immunoprecipitations with anti-δ or anti-CD3 indicates that mAbs 86D and 69C may not recognize all species of γδ TCR molecules. Only the anti-CD3 serum immunoprecipitated CD3 molecules, which migrated at ~19 – 21 kd (lane 4).

In ruminants a unique feature of γδ T cells is that they express a surface molecule termed T19, for which no human or mouse homolog has yet been defined (4,12; see below) This molecule is highly immunogenic in mice, and several mAbs to T19 have been produced. Figure 1, lanes 8 – 11, shows SDS – PAGE analysis of the bovine T19 molecule, immunoprecipitated using three different mAbs, 197, ILA-29, and 19-19. Under reducing conditions all mAbs immunoprecipitated the same sized molecule of ~215 – 220 kd. Under non-reducing conditions the T19 molecule migrated slightly faster, equivalent to ~200 kd (lane 11), indicating that the T19 molecule consists of a single polypeptide chain and most likely contains intra-chain disulfide bonds. The three antibodies almost certainly recognize the same 215 kd T19 molecule, since all three mAbs show an identical cellular reactivity, staining only γδ T cells (see below).

The large proportion of γδ T cells in bovine blood and the wide range of available surface markers (reviewed in 18) allowed us to assess the phenotype of bovine γδ T cells. Figure 2 shows the typical two-color immunofluorescence pattern observed. Expression of the 215 kd T19 molecule was restricted almost entirely to the 86D+ γδ subset. The very small number of T19+ cells that do not bind mAb 86D may represent γδ T cells which use a form of the receptor not recognized by this mAb. Also, a small percentage of γδ (86D+) T cells lacked the T19 molecule. Functional studies on T19- and T19+ γδ T cells in sheep have so far shown no differences between these subsets, although the T19- subset did preferentially localize to intestinal epithelium (12).

CD8 was expressed at low levels on ~10 – 20% of bovine 86D+ lymphocytes, and the proportion of CD8+ γδ T cells varied from animal to animal. CD4, CD2, and CD6 were expressed on only a very small proportion of γδ (86D+) lymphocytes (Fig. 2). The absence of the CD2 molecule from bovine γδ T cells is similar to results reported in sheep (12,19,20) and the implications of this for γδ T cell behavior have been discussed (19,20). The significance of CD6 expression on αβ but not γδ T cells is still unclear, since the function of this molecule has not yet been determined, although in humans and cattle it has been implicated in T cell activation (21,22).

We also analyzed γδ T cells for the expression of CD44, a molecule known to be involved in lymphocyte recirculation (23) since γδ T cells in sheep show a specific recirculation pattern (24). γδ T cells in cattle expressed only moderate levels of CD44 (Fig 2), lower than the levels found on CD4+ or CD8+ cells (not shown). It is therefore possible that the lower expression of CD44 on γδ T cells contributes to their relatively sluggish traffic through lymph nodes (see below).

We next analyzed the distribution of γδ T cells within skin, gut lymph nodes, and thymus, on the premise that this might clarify the cellular interactions and physiological significance of γδ T cells *in vivo*. Figure 3(a) shows a frozen section of bovine skin stained with the anti-γδ mAb 86D by the immunoperoxidase technique. γδ+ cells were present in large numbers, and were usually of an irregular shape with abundant cytoplasm, in contrast to γδ+ T cells in lymph nodes and thymus, where they were usually small and round. γδ T cells were localized within two areas of the skin. The majority accumulated as clusters within the dermis, sometimes close to the epidermis, and were also found interspersed between hair follicles. A minority of γδ+ cells were distributed within the epidermis, although not randomly, rather, they appeared to have infiltrated the epidermis at specific sites (Fig. 3a, arrows). In most instances the accumulations of γδ+ cells within the dermis merged with those in the epidermis. Staining of skin sections with anti-CD4 or anti-CD8 revealed few stained cells, indicating that the majority of γδ+ cells in skin were CD4-CD8-.

Within intestinal villi, γδ T cells were numerous and were present in both the lamina propria and the epithelium (Fig. 3b). γδ T cells within intestinal epithelium were mostly large, blast-like cells with an irregular shape. Most, if not all, intra-epithelial lymphocytes were CD8+. CD4+ lymphocytes were confined entirely to the lamina propria.

Lung tissue sections were stained with a panel of mAbs. Unlike skin or intestinal epithelium, γδ T cells comprised only a low proportion of all T cells, and showed no specific localization compared with CD4+ or CD8+ cells.

In bovine thymus the distribution of γδ T cells was identical to that previously described for sheep thymus (12). A remarkable feature of both bovine and ovine thymus was the large proportion of medullary thymocytes that were of the γδ type (Fig. 3c). This may reflect the high numbers of γδ T cells in peripheral blood of sheep and cattle, and so this feature may not hold for all species. A second feature was the characteristic clustering of γδ T cells around Hassall's corpuscles, which we have also shown in sheep (12), suggesting that Hassall's corpuscles may play a role in the maturation of γδ T cells, at least in these species.

We next examined cattle lymph nodes to see if γδ T cells were localized to any particular cellular compartment. Staining with mAb 86D revealed that γδ T cells were situated around the trabeculae which penetrate from the capsule into the node (Fig. 3d). γδ T cells also were localized to medullary sinuses but were usually absent from B cell follicles, whereas CD4+ cells were present in these sites (Fig. 3e), which probably relates to the helper role of CD4+ cells during T – B interactions. The T cell areas of the lymph node contained large numbers of CD4+ and CD8+ cells (Fig. 3e and f), but γδ T cells were sparse-to-absent

The absence of γδ T cells from B cell follicles was a general finding; however, in one of five lymph nodes examined, some γδ T cells were found within germinal centers.

The number of γδ T cells within lymph nodes was usually very low, much less than the numbers of CD4+ or CD8+ T cells, which is in contrast to the high proportion of γδ T cells found in the blood. This indicates that γδ T cells do not pass from blood to lymph nodes as freely as CD4+ or CD8+ cells. To demonstrate this more formally, we analyzed blood and lymph node cells from the same animals, and the typical reactivity pattern is shown in Table 1. γδ T cells within lymph nodes usually comprised 1 – 3% of cells in animals of 6 – 10 months of age, although higher values were seen in older animals. γδ T cells usually comprised 15 – 30% of PBL, although on one occasion 53% of cells were stained. The localization of γδ T cells in the lymph node, i.e. in areas adjacent to trabeculae and within medullary sinuses, suggests that the cells were purely in transit. Taken together with their low numbers, these findings imply that γδ T cells play little or no immunological role within lymph nodes.

The studies reported here in cattle provide further evidence that the numbers, phenotype, and distribution of γδ T cells can vary considerably from species to species. The presence of high numbers of γδ T cells in the skin of cattle and mice (7 – 9) is in marked contrast to the very low numbers found in the skin of humans (17), chickens (10), and sheep (unpublished). Secondly, the CD2 molecule, which promotes adhesion and activation of T cells, is expressed on circulating γδ T cells of humans (17) but not on those of cattle, sheep (12,19), or chickens (O. Vainio, personal communication). Thirdly, γδ T cells are distributed evenly throughout the human lymphoid system (17), whereas in cattle and sheep lymph nodes, these cells are concentrated around trabeculae and within the medulla. Fourthly γδ T cells show an association with Hassall's corpuscles within the thymic medulla of cattle and sheep, whereas no such association has been noted in humans (17). Lastly, in ruminants the γδ subset represents a numerous T cell type, particularly during the perinatal period of development (28). γδ T cells also represent a numerous T cell type in chickens (16) and in pigs (29; M. J. Reddehase and A. Saalmüller, personal communication), whereas humans and mice have low numbers of circulating γδ T cells.

Prominence of γδ T cells at epithelial surfaces is a feature which has been described in all species examined (7 – 12), although humans have relatively fewer γδ T cells at these sites (17). This conserved feature points towards the function of γδ T cells as a 'first line' defense for the elimination of infected or transformed epithelial cells (30). Our results certainly support this proposition, although it is not yet clear why species as diverse as cattle or sheep, chickens, and pigs have such high numbers of γδ T cells within the circulation. Environmental conditions could dictate that certain species need a more extensive repertoire for the γδ TCR. Alternatively, the function(s) of γδ T cells might overlap with those of αβ T cells, so that the relative proportions of these two T cell types in different species might be somewhat random and inconsequential. In any event, cattle join a growing list of species in which γδ T cells represent a numerous lymphoid cell type, which would indicate that γδ T cells are not just a minor redundant T cell subset.

The *in vivo* function of the γδ subset probably relates to those features of γδ T cells that are clearly distinct from αβ T cells. These include the recognition of antigen in an MHC-unrestricted fashion (12,13) or in the context of elements such as Qa-1 (15), the exclusive use of certain cell surface molecules such as T19, and the preferential localization to epithelial surfaces (7 – 12). A fuller analysis of the evolutionary history of γδ TCR and its usage among different species of animals, together with functional studies, may be required for a complete understanding of the physiological significance of γδ T cells in immune responses.

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Abbreviations

mAb	monoclonal antibody
MHC	major histocompatibility complex
PBL	peripheral blood lymphocyte(s)
SDS – PAGE	sodium dodecyl sulfate – polyacrylamide gel electrophoresis
TCR	T cell receptor

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