Antigen-specific immune response of porcine T lymphocytes to various pathogens

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Summary
The importance of swine in agriculture has resulted in a substantial increase in research efforts on the swine immune system during the past few years. A better knowledge of the porcine immune system is required before improved vaccination strategies, the design of more efficient vaccines and breeding for disease resistance will be able to contribute to a reduction in the extensive economic losses caused by the disabling effects of viral, bacterial and parasitic infections. T lymphocytes play a central role in the antigen-specific immune response to the various pathogens. To detect and characterise porcine T lymphocytes, monoclonal antibodies (mAbs) were raised against different leucocyte differentiation antigens and classified for specificity in two international workshops. These mAbs have enabled detailed studies to be made on specific cell populations involved in the porcine immune response to pathogens, on T lymphocytes and on the peculiaries of porcine T lymphocyte sub-populations: extra-thymic CD4+CD8+ T lymphocytes and a substantial proportion of CD2−TCRαβ+ T cells. Furthermore, these reagents and the increased knowledge of the immune system have allowed studies of the interactions of T lymphocyte sub-populations with regard to different pathogens and the role which these play in infections.

Keywords

Introduction
Studies on the porcine immune system have increased during recent years. In particular, the use of monoclonal antibodies (mAbs), which have been developed against distinct surface molecules of porcine leucocytes, has generated information on porcine leucocyte populations and their interactions (52, 80). These specific mAbs have altered the way in which the immune system is studied by allowing the identification of surface molecules on immune cells, as a result of which these cells can be classified, isolated and studied for their functional properties.

Current knowledge of porcine immune cell populations has facilitated comprehensive studies on the influence of infectious agents on the immune system and the interaction of these agents with distinct leucocyte populations (58, 59, 64, 65, 101). Understanding the interactions between immunogenic parts of infectious agents and the porcine immune cell populations is important with regard to further improvement of the efficacy of existing vaccines and the targeted development of new vaccines against various pathogens.

To facilitate such developments, extensive research must be performed to collect data on the non-specific immune response mediated by granulocytes and monocytes and on the characterisation of antigen-specific reactions of B and T lymphocytes, which are the most important aspects of swine immunology. There is a particular need to elucidate the role of porcine T lymphocytes, the specificity, variability and immunological memory of which contribute significantly to the functioning of the antigen-specific immune response.
Phenotypical characterisation of porcine T lymphocytes

Within a diverse panel of T-lymphocyte-specific surface antigens, CD3 (cluster of differentiation) molecules represent the most potent marker for the characterisation and definition of this leucocyte population (reviewed by Clevers et al. [26]). T lymphocytes express together with their respective T-cell receptor (TCR) CD3 molecules, which are important for signal transduction and activation of the respective T-lymphocyte clones (49) after recognition of peptide antigens presented by molecules encoded in the major histocompatibility complex (MHC) (reviewed by Kronenberg et al. [50]) by the specific TCR (1, 37, 48).

Prior to the description of mAbs directed against CD3 in swine in 1996 (72, 80, 110, 111), various approaches were used to define T lymphocytes with regard to other surface molecules (9, 12, 82, 83).

Within the porcine population of mononuclear leucocytes, T lymphocytes can be defined as thymus-derived cells expressing neither B-lymphocyte-specific antigens nor myeloid cell markers (82, 91). In contrast, mononuclear leucocytes belonging to the myeloid lineage are characterised, for example, by the expression of an antigen named SWC3 (66, 82, 83). The abbreviation SWC (swine workshop cluster) was determined during the First and Second International Swine Cluster of Differentiation (CD) Workshops (52, 80, 93), where SWC defines porcine antigens with no homologous molecules in other species. The nomenclature for antigens expressed on porcine leucocytes representing homologous molecules to human CD with regard to amino acid or DNA sequence is comparable to that used for human differentiation antigens. These antigens are also defined in swine as CD antigens (80, 93).

In addition to SWC3 positive mononuclear leucocytes belonging to the myeloid lineage, B lymphocytes form another prominent lymphocyte population. Porcine B lymphocytes are characterised either by their expression of surface immunoglobulins (4, 82) or by B-cell-specific non-immunoglobulin antigens, such as CD21 (29, 80). The porcine CD21 analogue should correctly be designated as wCD21. The antigen shows a high homology to the human CD21 with regard to surface expression and molecular mass (140 kiloDaltons [kDa]) (29), but in swine, the amino acid and DNA sequence data of the molecule are missing, so that wCD21 is the correct designation.

Efforts to characterise and separate porcine T lymphocytes have used different approaches. In 1977, Binns et al. were able to separate porcine B and T lymphocytes using sheep erythrocyte rosettes (11). By depleting B lymphocytes with anti-swine-immunoglobulin-conjugated sheep erythrocytes, T lymphocytes were enriched through their ability to bind to naive, untreated sheep erythrocytes. These experiments, based on the interaction of porcine CD2 molecules – the porcine sheep erythrocyte receptors (34) – with the sheep CD58, enabled the enrichment of the porcine T-lymphocyte population. Furthermore, Binns et al. were able to define a third lymphocyte population, which was negative for surface immunoglobulins and for CD2. This population was designated as null cells (11). Approximately ten years later, the null cells were characterised in further experiments as the CD2-negative and CD2-positive T lymphocyte fractions show surface expression of an antigen named SWC1 (81, 82, 83). B lymphocytes and activated T lymphocytes (81) are negative for SWC1, but SWC1 is not exclusively expressed on resting T lymphocytes, as cells of the myeloid lineage, monocytes and granulocytes also show a high antigen density of this surface marker (83, 90).

Porcine T lymphocyte sub-populations

Leucocyte differentiation antigens of vertebrates have been well conserved during evolution with regard to molecular structure, function and tissue distribution. With the exception of some porcine molecules mentioned above, which to date have no human or rodent counterpart (SWC antigens [10, 16, 52, 54, 84]), a large number of porcine leucocyte surface antigens represent analogous molecules of well-defined differentiation antigens of other species. However, cross-reactivity of the respective mAbs to these antigens is rare and is mostly limited to closely related species.

The function, tissue distribution and sequence of the porcine CD2 and CD3 molecules mentioned above seem to resemble those of other species. The porcine CD2 is able to bind xenogeneic sheep erythrocytes (13, 34). The molecule is characterised by a molecular mass of 48 kDa (84) and a murine antiserum directed against a highly conserved membrane region of the human CD2 (18) also recognises the porcine analogue (84).

Some of the mAbs directed against the porcine CD3 were produced using the corresponding eukaryotic and prokaryotic expressed gene product as antigen (45, 110). These mAbs detect a protein with a molecular mass of 23 kDa and were able to stimulate porcine T lymphocytes (44, 72). Both differentiation antigens CD2 and CD3 are indeed expressed on most of the Ig“SWC3” T lymphocyte population, but do not cover all cells included in this subset.

Expression of porcine CD4 and CD8

Porcine differentiation antigens CD4 and CD8 have been characterised with regard to molecular mass, tissue distribution, functional behaviour and involvement as
accessory molecules in the recognition of antigens presented by MHC molecules to the respective TCR. The porcine CD4 represents a monomeric glycoprotein of 55 kDa (66, 67, 70, 100), which is expressed on approximately 50% of thyocytes and a substantial subset of extrathymic peripheral blood T lymphocytes. Myeloid cells and B lymphocytes are negative for the CD4 antigen. Monoclonal antibodies directed against the CD4a epitope (80) are able to block MHC class II-restricted functional activity of porcine T helper cells (67, 99).

The porcine CD8 molecule, officially described as wCD8 (workshop CD8) due to a lack of amino acid and DNA sequence data, is a dimeric molecule with a molecular mass of 70 kDa under non-reducing conditions and 33 kDa-35 kDa under reducing conditions (41, 66). CD8 molecules are exclusively expressed on a substantial proportion of thyocytes and a subset of T lymphocytes. Monoclonal antibodies directed against the porcine CD8a or CD8b epitopes (88) are able to block MHC class I-restricted antigen-specific cytolytic T-cell activity (41, 67).

In the thymus, both CD4 and CD8 show an expression pattern comparable to that of other mammals (82, 92); apart from CD4+CD8- thymic progenitors and two fractions with the phenotype of more mature thyocytes, either CD4+CD8- or CD4+CD8+, the majority of the porcine thyocytes belong to a population showing co-expression of the two differentiation antigens with the phenotype CD4*CD8*, which have been defined in other species as common thyocytes (51, 96).

In the extra-thymic T lymphocyte compartment, the expression pattern of CD4 and CD8 differs completely from that known for other species (67, 82, 91, 113, 114). In addition to CD4+CD8- T lymphocytes with the phenotype of MHC class II-restricted T helper cells and CD4+CD8- T lymphocytes showing the phenotype of MHC class I-restricted cytolytic T lymphocytes, two further T lymphocyte sub-populations exist, namely:

a) double-negative CD4+CD8- T lymphocytes, which are common to swine and other ungulates, for example, sheep (55) and cattle (56), and which contain the majority of CD2+ TCR-γδ T lymphocytes in blood-derived populations

b) a substantial proportion of CD4+CD8+ double-positive extra-thymic T lymphocytes, which are unique for the porcine immune system (67, 71, 82).

These four sub-populations of extra-thymic T lymphocytes show high variance in proportion between different animals (82), and the percentages of CD4+CD8- and CD4+CD8+ seem to be inversely related (82). Studies conducted on T lymphocytes derived from different lymphoid tissues of one individual animal confirmed the existence of the four CD4/CD8-defined T lymphocyte sub-populations in lymphoid tissues, but also showed differences in the proportions of each group present in various lymphoid compartments (82, 88, 114). Furthermore, the ratio of double-negative to double-positive T lymphocytes seems to be age-dependent, with young animals showing a very high percentage of CD4+CD8- T lymphocytes and almost no CD4+CD8+ double positives, while older animals are characterised by an inverse ratio (82, 114). These findings are confirmed by studies with gnotobiotic animals which lack a CD4+CD8+ extra-thymic T-cell sub-population. These data demonstrate post-natal, extra-thymic, antigen-dependent changes in the porcine CD8 expression on T lymphocytes, which are combined with a further maturation and development of the respective porcine T lymphocyte sub-populations. This differentiation could be simulated in vitro, showing an extra-thymic generation of CD4+CD8+ T lymphocytes upon activation (82, 86, 113).

Phenotypical and functional characterisation of porcine T lymphocyte sub-populations

CD4+CD8- T lymphocytes

CD4+CD8- T lymphocytes represent a heterogeneous sub-population with regard to surface antigen expression. This group also shares specific surface antigens (for example, SWC6 recognised by mAb MAC320) (10, 14, 15) and most carry an antigen detected by mAb CC101 (20), which represents an analogue of the bovine WC1 (workshop cluster 1) (27, 108), but the group can also be phenotypically discriminated into various subsets.

The first antigen described which is exclusively expressed on the CD4+CD8- cell population is the SWC6 antigen, recognised by the mAb MAC320 (14). This antigen is characterised as a disulphide-linked heterodimer with a molecular mass of 270 kDa under non-reducing conditions and 150 kDa under reducing conditions, and is expressed on all CD2- T lymphocytes (14). In the peripheral blood-derived T lymphocyte population, most SWC6-positive cells co-express TCR-γδ-chains recognised for example by mAb PGBL22A, which seems to be directed against the porcine TCR δ-chain (28).

SWC6-positive TCR-γδ T lymphocytes contain subsets of cells which differ in expression of various TCR γ-chains (28, 35, 36, 74, 103) and of T-cell receptor independent surface antigens belonging to the workshop clusters SWC4 and SWC5. Variations in the molecular mass of the γ-chains enable the classification of three different TCR-γδ using biochemical methods (35, 36, 74). These data were confirmed on the molecular level, where three different constant γ-regions could be characterised (102, 103). In blood, most of the TCR-γδ T lymphocytes express TCR-γ-chains with a molecular mass of 37 kDa. More than one-third of the porcine peripheral blood TCR-γδ cell population shows expression of
an epitope on TCR-γδ, recognised by mAb 86D, which was developed against sheep TCR-γδ (57) and which shows cross-reactivity with part of the porcine TCR-γδ subset; these TCR-γδ are characterised by a molecular mass of 38/40 kDa (35). Furthermore, a third minor population of TCR-γδ T cells exists among peripheral T lymphocytes. Cells belonging to this population show the phenotype CD2+ and express TCR-γδ with a molecular mass of 46/40 kDa. This subset is enriched in lymphoid tissues and shows a homing behaviour which is different from that described for the other TCR-γδ subsets (40, 85). Besides variations in the TCR-γ-chains, CD2+ peripheral blood TCR-γδ T lymphocytes can be discriminated by the surface expression of differentiation antigens exclusively expressed by porcine CD2+ cells. These surface antigens were distributed in the past two CD workshops to the cluster SWC4 and SWC5 (15, 52, 80). The surface molecule included in the SWC4 cluster is characterised by a molecular mass of 280 kDa under non-reducing conditions and 160 kDa under reducing conditions (14), and represents a splicing variant of the SWC6 molecule (15). The antigen included in the SWC5 is a single chain protein of 150 kDa. Both clusters of surface antigens, SWC4 and SWC5, define partially overlapping cell subsets which are included in the SWC6-positive cell population (28).

The function of these null-cell specific differentiation antigens, as well as the function of the respective sub-populations and cell subsets defined by their expression, is still not clear and requires elucidation. Porcine TCR-γδ cells can be stimulated with mitogens and are able to show non-MHC-restricted cytolytic activity (A. Saalmüller, unpublished findings). However, to date nothing is known of the in vivo functions of these cells, the antigens recognised, the surface molecules involved in their functional capabilities and the phenotype of specific effector cells included in the heterogeneous sub-population of CD2+CD4+CD8+ TCR-γδ T lymphocytes.

**CD4+ T lymphocytes**

In contrast to the CD4+CD8+ TCR-γδ T lymphocytes, the porcine CD4 subset seems to be a more homogeneous T lymphocyte population. All porcine CD4+ T lymphocytes co-express CD2 (84), CD3 (111) and CD5 (89, 92) antigens. All CD4+ cells appear to belong to the TCR-αβ T lymphocyte subset. Interestingly, porcine CD4+ T lymphocytes can be discriminated by their CD8 expression into two sub-populations (67, 82). In contrast to all other species, a substantial proportion of CD4+ extra-thymic T lymphocytes express CD8 antigens (67, 82); therefore, two sub-populations of extra-thymic CD4+ T lymphocytes exist: CD4+CD8- cells with the phenotype of classical T helper cells, and CD4+CD8+ T lymphocytes, which are unique to the porcine immune system. CD4+CD8+ extra-thymic T lymphocytes certainly show the phenotype of CD4+CD8+ common thymocytes but differ from thymocytes, both morphologically and with regard to other differentiation antigens: CD4+CD8+ extra-thymic T lymphocytes show no expression of the thymocyte-specific CD1 antigen, in contrast to CD4+CD8+ thymocytes (69, 84). Furthermore, the extra-thymic CD4+CD8+ T lymphocytes have the morphological phenotype of mature and resting T lymphocytes, as shown in histology and electron microscopy (86, 91). Functionally, both CD4+ T lymphocyte sub-populations are able to respond to mitogen and to alloantigen in mixed leucocyte cultures (MLC). An MHC class II-restricted proliferative immune response and synthesis of cytokines (interleukin-2/4) are induced in both sub-populations by stimulation with alloantigenic irradiated leucocytes (99). All CD4+ T lymphocytes show a T helper cell function for the generation of alloantigen-specific cytolytic T lymphocytes and T-cell-dependent in vitro synthesis of immunoglobulins (A. Saalmüller, unpublished findings). In contrast to polyclonal stimulation with mitogen and oligoclonal activation in the MLC, which represent primary in vitro immune reactions, the two CD4+ T lymphocyte sub-populations differ completely regarding secondary antigen-specific responses to recall antigen (99). In contrast to the primary immune response, where both T helper sub-populations are able to react, only the CD4+CD8+ T lymphocytes show a significant antigen-specific secondary immune response (99). This reaction is MHC class II-restricted and the additionally expressed CD8 molecules seem to maintain no CD8-specific functional activity (99).

**CD4-CD8+ T lymphocytes**

The porcine CD4-CD8+ T lymphocyte sub-population contains two main subsets of lymphocytes which can be discriminated according to immunological functions; one subset can be defined by spontaneous cytolytic activity against xenogeneic tumour cells, for example (68); the other subset includes progenitors of MHC class I-restricted cytolytic T lymphocytes (41). Cytolytic T lymphocytes (CTLs) derived from these progenitor cells can be directed against either alloantigen-specific target cells after stimulation with alloantigen in MLC (41, 65, 81, 92) or, in a secondary in vitro immune response, against virus-infected autologous target cells (58, 64, 65).

The cells included in the CD4-CD8+ T lymphocyte sub-population are characterised by a heterogeneous CD8 antigen expression with a CD8 surface antigen density ranging from very low to high (65, 82, 88). Unique to all CD8+ cells is the co-expression of CD2 molecules (84, 91). With regard to other differentiation antigens, the CD8+ sub-population can be discriminated into at least two subsets according to the expression of CD3 (111), CD5 (92) and CD6 (65). CD4-CD8+ cells with low CD8 antigen density express neither CD5 (89, 92) nor CD6 (65, 87) antigens. Moreover, the majority of these cells are also negative for CD3 (111). This absence of expression of distinct molecules involved in typical T-cell activities indicates a possible function of the subset. The CD3-CD5-CD6-CD8low cell population is responsible for spontaneous non-MHC-restricted cytolytic
activity within the CD8^+ lymphocyte sub-population (65, 92). Monoclonal antibodies directed against MHC class I and CD8 molecules are unable to block this cytolytic activity (68). In contrast to the CD8^{low+} cells, CD8^+ cells with high CD8 expression show a distinct co-expression of CD3 (111), CD5 (89, 92) and CD6 (65, 87) molecules. This cell fraction includes the progenitors of the alloantigen-specific CTLs (92), as well as virus-antigen-specific CTL (64, 65). The cytolytic activity of both types of CTL can be blocked by the addition of mAbs directed against MHC class I molecules and/or CD8a or CD8b epitopes (41, 67, 88). This confirms the functional difference to the CD8^{low+} cell population and shows that the CD3^{CD5+CD6+CD8^{high+}} T-cell fraction contains the MHC class I-restricted cytolytic T lymphocyte subset.

Porcine T-cell response to various pathogens

The number of studies conducted on porcine cellular immune responses to various viral (25, 59, 94), bacterial (105) and parasitic (6) pathogens is still limited, in comparison with work performed on other species. In the past, most studies on interactions between pathogens and the porcine immune system could only suggest the participation of antigen-specific, MHC-restricted T lymphocytes in the respective immune responses, and the subject was discussed without final proof being obtained (97, 98, 106, 109). Nevertheless, the number of studies performed on the antigen-specific immune response of swine has increased in recent years and specific reagents, improved technology and a more detailed knowledge of the porcine immune-cell populations now enable both an enhanced alignment of the interactions between pathogens and the porcine immune system and detailed analyses of the antigen-specific T-cell response.

Antigen-specific immune response to bacteria

Information on the reactivity of porcine cellular immune responses to bacterial antigens is still scant. There is little documentation on the reactivity of T lymphocytes and the respective T lymphocyte sub-populations to bacterial antigens. The local immune response has been studied and described for most bacterial infections. T-cell activity was detected by an enhanced migration and infiltration of activated CD4^+ and CD8^+ T lymphocytes into the infected perivascular and peribronchial regions of the lung (8). In *Actinobacillus pleuropneumoniae*-infected animals, a blastogenic in vitro response of peripheral lymphocytes was described, although the phenotype of the responding T lymphocyte sub-populations was not defined (32).

Antigen-specific immune response to parasitizes

Parasite-specific T-cell response has been studied for *Ascaris suum* (53) and *Trichinella spiralis* (30, 39). In *A. suum*-infected swine, an increase in the macrophage population was detected, whereas the level of T helper cells and cytolytic T cells did not change after exposure to the parasite. An interesting finding was an up-regulation of MHC class II antigen expression on cells of the immune system, indicating antigen-specific activation (53). An increase in the number of MHC class II^+ peripheral blood mononuclear cells was also detected in pigs which had been exposed to *T. spiralis* (39). During the course of infection, infected animals also showed a persistent elevation in the number of both CD4^+ and CD8^+ T lymphocytes (39). T cell lines from pigs inoculated with *T. spiralis* developed antigen-specific, cytokine-dependent proliferation in response to *T. spiralis* stimulation in vitro. The majority of the responding activated T lymphocytes expressed both CD4 and CD8 antigens. In addition, these T lymphocytes could be characterised by the expression of CD25 (interleukin-2 receptor [IL-2R]) (5) and MHC class II surface antigens. These results indicate that CD4^+CD8^+ T lymphocytes are involved in the anamnestic cell-mediated immune response to *T. spiralis* (30).

Antigen-specific immune response to viral antigens

Current knowledge of the reactivity and phenotype of the porcine T lymphocyte sub-populations against pathogens was obtained by studying virus infections and the response of the porcine immune system to viral antigens.

To date, the involvement of porcine T lymphocytes in the anti-viral immune response has been demonstrated mostly by T lymphocyte stimulation experiments, in which T lymphocytes derived from infected animals are isolated and restimulated in vitro with the respective antigens (2, 3, 17, 19, 23, 42, 43, 76, 95, 99, 106, 107). In some viral infections, the influence of the cellular defence mechanisms has been studied in greater detail. All data described to date are summarised in Table 1.

In animals which had recovered from African swine fever virus (ASFV) infection (a viral disease caused by a double-stranded DNA virus belonging to the family *Iridoviridae*), virus-specific T-cell reactivity could be demonstrated for
by apoptosis (73). In addition to these apoptotic events, for lymph node cell depletion and intense lymphoid cell necrosis monocytes decreased in peripheral blood correlating with ASFV, in particular, the levels of B lymphocytes and immune response. In acute infections with highly virulent virus-specific T lymphocytes with the T helper function and MHC class I-restricted cytolytic activities seem to be the important element of the immune system in anti-ASFV immune response. In acute infections with highly virulent ASFV, in particular, the levels of B lymphocytes and monocytes decreased in peripheral blood correlating with lymph node cell depletion and intense lymphoid cell necrosis by apoptosis (73). In addition to these apoptotic events, for ASFV strains of more moderate virulence, modulation and immune response have inefficient anti-viral effects, virus-specific T helper cells which have been identified as being involved in the ASFV-specific immune response (58).

Table I
Summary of the reactivity of porcine T lymphocytes to different viruses

<table>
<thead>
<tr>
<th>Reactivity of porcine T lymphocytes</th>
<th>African swine fever virus</th>
<th>Classical swine fever virus</th>
<th>Foot and mouth disease virus</th>
<th>Pseudorabies (Aujeszky's disease) virus</th>
<th>Porcine reproductive and respiratory virus</th>
<th>Transmissible gastroenteritis virus</th>
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<tr>
<td>All T lymphocytes</td>
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<td>T helper cells</td>
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<td>Cytokine production</td>
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<td>+</td>
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<td>Help in immunoglobulin synthesis</td>
<td>+</td>
<td>NA</td>
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<td>Cytolytic cell subsets</td>
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<td>Cytolytic T lymphocytes</td>
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<td>Natural killer cells</td>
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<td>Phenotype of the reactive T-cell population(s)</td>
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<tr>
<td>CD4+</td>
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<tr>
<td>Characterisation of T-cell epitopes</td>
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<td>MHC-I peptides</td>
<td>MHC-II peptides</td>
<td>MHC-I/MHC-II</td>
<td>MHC-I/MHC-II</td>
<td>MHC-I/MHC-II</td>
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NA : not available
CD : cluster of differentiation antigens
MHC-I : major histocompatibility complex class I
MHC-II : major histocompatibility complex class II

T helper cells (19, 22, 75) and for CTLs (58). In this viral disease, where the B-cell compartment and the humoral immune response have inefficient anti-viral effects, virus-specific T lymphocytes with the T helper function and MHC class I-restricted cytolytic activities seem to be the important element of the immune system in anti-ASFV immune response. In acute infections with highly virulent ASFV, in particular, the levels of B lymphocytes and monocytes decreased in peripheral blood correlating with lymph node cell depletion and intense lymphoid cell necrosis by apoptosis (73). In addition to these apoptotic events, for ASFV strains of more moderate virulence, modulation and down-regulation of porcine MHC class I and MHC class II antigen expression on infected macrophages were demonstrated in vitro (33), as was the severe inhibition of the natural killer cell activity of porcine peripheral blood mononuclear cells co-incubated with ASFV (60). The induction of different interferons – interferon-α (IFN-α) and interferon-γ (IFN-γ) – might reduce the replication of the virus in blood monocytes and alveolar macrophages (21, 31), but these effects seem to be less significant than the T-cell activity. Animals which survived infection caused by moderate or attenuated low virulence ASFV strains showed a strong virus-induced T-cell response which was visible by blast transformation and proliferation (95, 106), IL-2 and IFN-γ production (75). CD8+ and CD4+ T lymphocytes are stimulated in ASFV-antigen specific MHC class I and MHC class II-restricted reactions (19). In addition to the proliferative response and T helper cell activities described above, virus-specific CD8+ MHC class I-restricted CTLs could also be identified as being involved in the ASFV-specific immune response (58).

Virus-specific MHC-restricted CTLs also appear to play a role in the porcine immune response to foot and mouth disease virus (FMDV) (a small RNA-virus belonging to the family Picornaviridae). An FMDV-specific CD8+ MHC class I-restricted T-cell clone has been isolated from a vaccinated animal (78). Studies using polyclonal CTLs derived from FMDV-infected inbred swine showed specific reactivity to virus-specific antigens belonging to non-structural proteins (A. Saalmüller, unpublished findings), whereas FMDV-specific T helper cell epitopes were mainly located in the structural proteins, especially viral protein 1 (VP1) and VP3 (76, 77). Virus-specific T helper activities could be shown by virus-specific proliferative responses (76), by synthesis of IL-2 and IFN-γ (77) or by T-cell-dependent in vitro synthesis of FMDV-specific immunoglobulins (77).

In animals infected with classical swine fever virus (CSFV) ( hog cholera virus), protection against lethal infection is correlated with a humoral immune response resulting in the production of virus-specific antibodies directed against structural proteins (38, 46, 79). This B-cell response strongly suggests a contribution of T helper cells, which has been confirmed by the quantification of virus-specific proliferation of CD4+ T lymphocytes (42). In addition to the MHC class II-restricted T helper cell response to CSFV, the involvement of virus-specific CTLs in the cellular immune response to CSFV is well characterised. Cytolytic T lymphocytes derived from CSFV-infected dd-haplotype inbred swine recognise an MHC class I-restricted CTL epitope located on a non-structural protein (64). This was the first CTL epitope to be described in swine which was characterised by a nona-peptide sequence (64).

Cytolytic immune response mechanisms against pseudorabies virus (PRV) ( an alphaherpesvirus which causes Aujeszky's disease) was demonstrated by different groups defining two glycoproteins as the major target antigens (61).
Glycoprotein B (gB) was identified as the important antigen for MHC class I-restricted CTLs (112), whereas gB and gC seemed to be the immunorelevant structures for the non-MHC-restricted cytolytic response mediated by natural killer (NK) cells and lymphokine activated killer (LAK) cells (44), which have been characterised as CD2⁺CD4⁻CD8⁺ lymphocytes. The reactivity of porcine T lymphocytes to PrV determined in virus-antigen-specific proliferation assays confirmed the importance of the two glycoproteins gB and gC as parts of the virus envelope showing the major T-cell stimulating activity (43). Both CD2⁺CD4⁺ and CD2⁺CD8⁺ T lymphocytes were identified as responder cell populations (43). Studies with separated T lymphocyte sub-populations classified the PrV-specific MHC class II-restricted T helper cell memory response to the CD4⁺CD8⁺ T lymphocyte sub-population (99), but CD4 single positive cells might also be able to respond in the acute phase of the infection (114).

Swine infected with porcine reproductive and respiratory syndrome virus (PRRSV) showed blastogenic response and virus-specific antibody production (104) which might result from the activation of virus-specific CD4⁺ T helper cells. Experiments demonstrated that the CD4⁺ sub-population indeed contained the majority of antiviral effector cells (7).

Virus-specific T lymphocyte reactivity has also been described for two porcine coronaviruses, namely: transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (94), both of which induce proliferative responses in T lymphocytes derived from mesenteric and bronchial lymph nodes and show a cross-reactivity in T-cell stimulation capacity (17). Virus-specific T helper cell activity showing interferon production (62) and in vitro synthesis of TGEV-specific antibodies (63) have also been demonstrated with peripheral T lymphocytes. Four MHC class II-restricted T helper cell epitopes characterised by their amino acid sequence were localised on the nucleoprotein (N protein) and on the membrane (M) protein (2), and all peptides initiated T helper cell activity for the production of virus-specific antibodies (2, 3). In addition to this clearly defined T helper cell activity, the existence of cytolytic cell fractions was also demonstrated (24, 98). However, the phenotype of these cytolytic cell fractions and their possible MHC restriction, if an MHC-restricted cytolytic activity is shown at all, remain unclear.

Conclusions

The results of different approaches used to study the interactions between porcine T lymphocytes and bacterial, parasitic and viral pathogens have provided scarce information to date. In parasitic infections, CD4⁺CD8⁺ T lymphocytes were shown to be involved (30), whereas in bacterial infections, activated CD4⁺ and activated CD8⁺ cells were seen to migrate to the respective bacterial infected tissues (8, 47). Most knowledge of the interactions between T lymphocytes and pathogens in swine has been generated by the study of viral infections. T lymphocytes involved in anti-viral activities of the porcine immune system showing T helper cell activity belong to the CD4⁺ (2, 3, 19, 42, 43, 75, 76, 77, 114) and CD4⁺CD8⁺ (99, 114) T-cell sub-populations. For responding CTLs, these could be defined as CD8⁺ MHC class I-restricted CTLs (58, 64, 65, 112) and CD8⁺ cells with NK or LAK cell activity (44). Some interaction between viral antigens and the respective T lymphocyte sub-populations has been characterised, at least in MHC-defined inbred animals, at the molecular level (2, 64). These experiments have led to the identification of MHC class I (64) and MHC class II restricted (2) viral T-cell epitopes. On the other hand, this information, which might be important for more detailed analyses of the interactions between the porcine immune system and pathogens, is still lacking for the majority of porcine viruses.

The area of veterinary immunology concerned with the molecular mechanisms of such interactions will be particularly important in the development of future research aimed at controlling and eradicating a wide variety of infectious diseases of swine. These studies will facilitate the improvement of existing immune modulating drugs and vaccines and will also allow the design of new vaccines on the molecular level. These achievements will lead to a better understanding of the porcine immune system, to improved animal health and a higher quality of products derived from these animals.

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Lymphocytes T et réponse immune spécifique de l’antigène à divers agents pathogènes chez les porcins

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Résumé
Du fait de l’importance de l’élevage du porc, les travaux de recherche sur le système immunitaire de cette espèce se sont considérablement développés au cours des dernières années. L’amélioration des stratégies de vaccination, la mise au point de vaccins plus efficaces et la sélection génétique pour une meilleure résistance aux maladies – tous éléments qui pourraient contribuer à réduire les graves pertes économiques dues aux infections virales, bactériennes et parasitaires et à leurs effets néfastes – impliquent une connaissance plus approfondie du système immunitaire porcin. Les lymphocytes T jouent un rôle majeur dans la réponse immune, spécifique de l’antigène, aux divers agents pathogènes. Pour déceler et caractériser les lymphocytes T porcins, des anticorps monoclonaux ont été dirigés contre divers antigènes de différenciation des leucocytes et classés, selon leur spécificité, à l’occasion de deux ateliers internationaux. Ces anticorps monoclonaux ont permis d’effectuer des études détaillées sur des populations cellulaires spécifiques intervenant dans la réponse immune des porcins aux agents pathogènes, sur les lymphocytes T ainsi que sur les particularités de sous-populations de lymphocytes T porcins: lymphocytes T extrathymiques CD4⁺CD8⁺ et une proportion importante de cellules CD2⁺TCRγδ⁺ T. Grâce à ces anticorps et à une meilleure connaissance du système immunitaire, il est maintenant possible d’étudier les interactions des sous-populations de lymphocytes T avec divers agents pathogènes ainsi que leur rôle au cours de l’infection.

Mots-clés

Linfocitos T y la respuesta inmune específica de antígeno contra patógenos varios en el cerdo

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Resumen
La gran importancia agropecuaria del cerdo ha propiciado en los últimos años un notable incremento de las investigaciones dedicadas al sistema inmunológico de este animal. La elaboración de estrategias de vacunación más adecuadas, la producción de vacunas más eficaces y la creación selectiva de animales más resistentes a las enfermedades, no podrán contribuir a una reducción de las enormes pérdidas económicas derivadas de los daños efectos de infecciones víricas, bacterianas o parasitarias, sin un mejor conocimiento previo del sistema inmunológico porcino. Los linfocitos T desempeñan un papel central en la respuesta inmune específica de antígeno contra diversos patógenos. Con objeto de detectar y caracterizar linfocitos T porcinos, se generaron anticuerpos monoclonales contra distintos antígenos de diferenciación de leucocitos, que fueron luego clasificados según su especificidad en el curso de dos talleres internacionales. Estos anticuerpos monoclonales han permitido estudiar en detalle tanto determinadas poblaciones celulares que intervienen en la reacción
inmune porcina contra organismos patógenos como los linfocitos T y los rasgos característicos de las subpoblaciones porcinas de linfocitos T: linfocitos T CD4⁺CD8⁻ extratímicos y una considerable proporción de células T CD2⁺TCRγδ⁺. Esos reactivos, por otra parte, sumados al creciente conocimiento del sistema inmunológico, han permitido estudiar las interacciones de subpoblaciones de linfocitos T en relación con distintos patógenos, así como la función que desempeñan durante las infecciones.

**Palabras clave**

**References**


