A CYTOTOXIC MURINE MONOCLONAL ANTIBODY THAT RECOGNIZES A PORCINE T CELL SUBSET INVOLVED IN LECTIN-INDUCED PROLIFERATION

Kw Kelley, H Salmon, Wc Davis, J Aynaud, A Paraf

Ann Rech Vét 1988 19:141-147

A CYTOTOXIC MURINE MONOCLONAL ANTIBODY THAT RECOGNIZES A PORCINE T CELL SUBSET INVOLVED IN LECTIN-INDUCED PROLIFERATION

KW KELLEY[1], H SALMON[2a], WC DAVIS[3], J AYNAUD[2] and A PARAF[2]

1 : Laboratory of Immunophysiology, Department of Animal Sciences, University of Illinois, 162 ASL, 1207 West Gregory Drive, Urbana, IL 61801, USA

2 : Institut National de la Recherche Agronomique, Laboratoire de Pathologie Porcine, Centre de Tours, Nouzilly, 37380 Monnaie, France

3 : Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99163, USA

received 07/09/87, accepted 03/11/87

Résumé

IDENTIFICATION PAR UN ANTICORPS MONOCLONAL MURIN D'UNE SOUS-POPULATION LYMPHOCYTAIRE T IMPLIQUÉE DANS LA PROLIFÉRATION INDUITE PAR LES LECTINES CHEZ LE PORC. – Une collection d'anticorps monoclonaux cytotoxiques a été obtenue à partir de souris immunisées avec des thymocytes de porc. Ces anticorps monoclonaux réagissent avec des déterminants monomorphes des lymphocytes sanguins de porc. Ils reconnaissent de 25 à 100 % des lymphocytes sanguins par le test de microfluorimétrie de flux. Un de ces anticorps monoclonaux (PT 81) qui se fixe sur 39 % des lymphocytes sanguins, 30 % des splénocytes et 65 % des thymocytes (déterminé par microfluorimétrie de flux) a été choisi pour une première caractérisation. PT 81 lyse spécifiquement 37 % des lymphocytes sanguins préparés sur Ficoll/Hypaque puis épuisés en monocytes par ingestion de fer carbonyl. Cet anticorps lyse 47 % des cellules T (lymphocytes sanguins qui forment des rosettes avec les globules rouges de mouton, augmentées par le dextran ; $P < 0,001$) et 64 % d'une sous-population de cellules T (lymphocytes sanguins qui forment des rosettes avec les globules rouges de porc, augmentées par le dextran, $P < 0,001$) en comparaison aux pourcentages obtenus avec des cellules traitées par le complément seul. La lyse des lymphocytes sanguins par PT 81 plus complément provoque un enrichissement de 100 % du nombre des cellules ayant des immunoglobulines de surface. La conclusion que PT 81 ne reconnaît pas les cellules B a été confirmée ultérieurement par des expériences de double marquage. L'épuisement des cellules PT 81 ne modifie pas de manière significative le nombre de cellules ayant les récepteurs C3b (rosettes zymosan-complément) ou les récepteurs Fcγ (rosettes globules rouges-anticorps). Les lymphocytes sanguins, traités avec PT 81 plus complément, ont été centrifugés sur Ficoll/Hypaque (densité : 1,09) pour enlever les cellules mortes. La population de lymphocytes épuisés ne montre que 20 %, 20 %, 26 % et 29 % de la réponse proliférative des cellules témoins traitées avec le complément seul ($P < 0,01$) à plusieurs concentrations de mitogènes : respectivement la phytohemagglutinine, la concanavaline A, l'agglutinine de soja et le mitogène de *Phytolacca* (pokeweed). L'incubation de l'anticorps PT 81 avec des lymphocytes sanguins pendant la coculture avec les mitogènes, en absence de complément, n'affecte pas la réponse proliférative. Ces résultats montrent que l'anticorps monoclonal PT 81 ne marque pas les cellules B mais reconnaît un épitope présent seulement sur une sous-population de cellules T, et que cette sous-population de cellules T est essentielle pour obtenir une réponse proliférative aux lectines.

Lymphocytes of pigs have been divided into two major subsets by the presence of surface membrane immunoglobulin (Ig ; B cells) and T cell antigens (T cells ; Salmon 1978). Various rosette assays have led to a further division of these cells by different receptors (Salmon 1979) : (1) sheep erythrocyte rosettes (2) a subset of sheep erythrocyte rosettes which bind pig erythrocytes (the so-called autologous rosette formation) (3) the third component of complement, C3b (4) Fcγ and (5) null cells that do not bind to any reagent. However, rosette formation does not allow division of T cells into classical CD4 (helper/inducer) and CD8 (cytotoxic/suppressor) cells. Recently, Pescovitz *et al* (1984, 1985) and Jonjic and Koszinowski (1984) succeeded in employing monoclonal antibodies (Moab) to identify CD4 and CD8 cells in pigs. A Moab to the porcine sheep erythrocyte receptor (CD11) has also been produced (Hammerberg and Schurig 1986).

a : Reprints request to H. Salmon.

Table 1. – A panel of murine Moabs that recognize varying proportions of porcine PBL, as determined by flow microfluorimetry.

Monoclonal Antibody	Isotype	Cells labelled (%)
PT 9 A	IgG2a	35
PT 15 A	IgG2a	75 - 85
PT 25 D	IgG3	85 - 100
PT 35 A	IgG3	85 - 100
PT 36 B	IgG1	32
PT 37 A	IgG3	45
PT 40 A	IgG3	85 - 100
PT 80 B	IgG2a	75 - 85
PT 81	IgG2b	45
PT 85 A	IgG2a	100
PT 91 A	IgG3	25

In this report, we describe the development of a panel of Moabs directed at monomorphic determinants on porcine peripheral blood lymphocytes (PBL). One of these Moabs was characterized and found to specifically label and kill 37 % of PBL in the presence of rabbit complement. This Moab did not recognize B cells, lysed 47 % of PBL that formed rosettes with sheep erythrocytes and significantly reduced the proliferative response of PBL to T cell mitogens. This antibody and others in this panel should be useful in delineating the processes involved in lymphoid cell differentiation and function in an economically-important outbred species.

Materials and Methods

Experimental Animals. All pigs used in these studies were crossbred, castrated males or females of either Hampshire × Yorkshire or Large White × Landrace breeds. Pigs were between 3 to 12 months of age.

Lymphoid Cell Preparation. Thymocytes were prepared from several porcine thymic lobes by repeated flushings with a tuberculin syringe and 25 gauge needle into sterile Dulbecco's modified Eagle medium (DMEM ; GIBCO Laboratories, Grand Island NY) with supplemental penicillin (100 units/ml), streptomycin (100 mg/ml) and glutamine (2 mM). PBL were prepared from heparinized whole blood. For the rosette and mitogenesis experiments, phagocytic cells were depleted (Goldstein *et al* 1973, Buschman and Pawlas 1980) by incubation of whole blood with carbonyl iron at 37 °C for 1 hour with constant rotation, as described previously (Salmon 1979). Blood was then diluted at a ratio of 1 part blood to 2 parts phosphate buffered saline (PBS) and layered over Ficoll/Hypaque (density : 1.09) in polystyrene 50 ml tubes (Westly and Kelley 1984). When necessary, erythrocytes present in cells at the plasma interface were lysed with a solution of 0.155 M NH_4Cl–0.01 M $KHCO_3$–0.01 M EDTA. Before use, cells were washed three times at 400 × *g* for 10 min at 4 °C in RPMI 1640 with supplemental penicillin, streptomycin and glutamine.

Preparation of Moab Against Cell-Surface Leukocyte Antigens. Six-to-10-week-old B10.A(3R)(H-2^{5i}) × Balb/c (H-2^{d}) mice were immunized twice ip and im with 5-10× 10^6 thymocytes. The first immunization was in an equal volume of Freund's complete adjuvant, and the second immunization was with incomplete adjuvant. Three days prior to fusion, mice were injected iv with 5 × 10^6 thymocytes. Single cell suspensions of splenocytes from immunized mice were prepared, pelleted for 10 min at 400 × *g* at 4 °C and washed two times with DMEM. A total of 10^8 spleen cells were pelleted and mixed 4 × 10^7 P3X63-Ag8.653 plasmacytoma cells and pelleted. The fusion was carried out in one milliliter of 50 % polyethylene glycol 1540 (JT Baker Chemical Co, Phillipsburg NJ). Ten milliliters of DMEM were slowly added over a 10-min period. Fused cells were pelleted and diluted with fresh, irradiated (2 000 RAD) Balb/c thymocytes at 5 × 10^6/ml in HAT (hypoxanthine-aminopterin-thymidine) prepared in full DMEM (13 % fetal bovine serum and 5 × 10^{-5} M 2-mercaptoethanol). Fused cells were distributed in 200 µl aliquots to ten 96-well culture plates (Costar, Cambridge MA). Hybridomas were maintained in HAT for 2 weeks before being changed to full DMEM, with feeding of cultures every 3 to 5 days.

Positive supernatants containing antibodies for leukocyte antigens were detected by screening supernatants at two weeks after fusion by a standard complement-mediated microcytotoxicity assay. Tissue culture supernatants (2 µl) were added to Terasaki plates previously prepared with 8 µl mineral oil per well. Two microliters of PBL at 3 × 10^6/ml were added. The plates were vortexed and incubated at room temperature for 20 min. Prescreened rabbit complement of low toxicity was diluted 1:4 and then added (2 µl) to each well, and the plates were vortexed and incubated at 37 °C for 30 min. Four microliters of 1 % eosin diluted in PBS were added to each well, incubated for 1 min, and 4 µl of 4 % formalin in PBS were added to fix the cells. All plates were scored for cytotoxicity with an inverted, phase contrast microscope (100 x) using the standard NIH scoring system. For experiments with PT 81, viable cells were counted and backgrounds were determined as the percentage lysis in the presence of complement alone. Percent specific cytotoxicity was calculated according to standard methods (North 1980). Positive cultures were transferred to 24-well plates and maintained in static culture by periodic thinning for two weeks and then retested. Positive cultures were expanded in 6-well culture plates and then frozen in liquid nitrogen. Hybridomas were cloned by limiting dilution in the presence of thymic feeder cells. Immunoglobulin

Table 2. – Distribution of cells that are recognized by Moab PT 81.

Tissue	Positive Cells (%)	
	Culture Medium	PT 81
Thymus	0.6 ± 0.1	66 ± 4
PBL	8.0 ± 2.0	47 ± 2
Spleen	8.0 ± 2.0	38 ± 2

The second antibody was a fluoresceinated F(ab')$_2$ rabbit anti-mouse heavy and light chain immunoglobulin. Values are the average of four 3-month-old pigs, ± SEM.

isotypes were determined by Ouchterlony analysis with isotype specific antisera (Becton-Dickinson, Sunnyvale CA).

Flow Microfluorimetry. Supernatants from hybridomas of interest were tested on PBL from 3 to 5 pigs with a FACS™ analyzer (Becton-Dickinson, Sunnyvale CA). PBL were adjusted to 10^7 cells/ml in DMEM. Cells (50 µl) were incubated for 30 min at 4 °C with 50 µl of culture supernatants or fresh medium. PBL were washed three times at 4 °C with PBS, 0.5 % BSA, 0.1 % sodium azide and incubated for 30 min at 4 °C with 50 µl of a 1/100 dilution of fluoresceinated goat anti-mouse immunoglobulin (Tago, Burlingame, CA). Cells were washed two additional times, fixed in 2 % formalin-PBS and analyzed by counting 5×10^3 cells on logarithmic amplification. The Moab PT 81 was characterized on lymphoid cells from blood, thymus and spleen using an EPICS V (Coulter Corporation, Hialeah FL) in combination with a fluoresceinated F(ab')$_2$ rabbit anti-mouse heavy and light chain immunoglobulin fragment (Tago Burlingame CA) as the second antibody.

Rosetting Test and Surface Immunoglobulin. Rosette tests were performed to determine the type of cell that was depleted after lysis with PT 81 in the presence of complement. All tests were conducted under optimal rosetting conditions, as previously described (Salmon 1979, 1982). T cells were detected with dextran-enhanced, sheep erythrocytes : this test gave similar results as anti-T cell serum (Salmon 1978). A subset of T cells was measured with dextran-enhanced, porcine erythrocytes. Fcγ positive lymphocytes were detected with the IgG fraction of porcine anti-bovine erythrocyte antiserum used at a subagglutinating dose with bovine erythrocytes (the EA rosette). Lymphocytes bearing receptors for the C3b component of complement were measured with complement-activated zymosan. Finally, membrane-surface immunoglobulin (Ig) was measured with a fluoresceinated F(ab')$_2$ goat anti-pig heavy and light chain immunoglobulin fragment using techniques described for flow microfluorimetry. Fluorescent cells were enumerated using a light microscope with Ploem equipment.

Double Fluorescence Labelling. To determine if the epitope identified by PT 81 was present on B cells, porcine splenocytes were incubated with rabbit IgG (1 mg/ml) to block binding of F_c receptors on porcine splenocytes. Surface Ig positive cells were determined with a rabbit anti-pig IgG heavy and light chain TRITC-labelled antibody (Zymed Laboratories, South San Francisco, CA). PT 81 positive cells were identified using indirect immunofluorescence with a fluoresceinated F(ab')$_2$ fragment as described above. Fluorescence was determined with a Zeiss Photomicroscope III with an excitor-barrier filter and reflector combination for FITC and TRITC (Davila *et al* 1987).

Removal of PT 81 Positive Cells by Cytotoxicity. Preliminary experiments with PT 81 supernatant from grow-to-die tissue culture flasks indicated that the antibody titer for cytotoxicity was 1:640. Therefore, a final dilution of 1:160 was used along with rabbit complement at a final dilution of 1:4. Removal of cells by complement-mediated cytotoxicity was conducted as previously described (Salmon 1978). Dead cells were removed by centrifugation over a Ficoll/Hypaque mixture (density 1.09) using methods similar to the purification of PBL, except that the lymphocyte suspension that was placed on Ficoll/Hypaque was pre-treated at 37 °C to warm the cells to room temperature. Cells were washed three times and viability of cells in the three treatment groups (original cells, original cells plus complement alone and original cells plus complement and PT 81) was determined by trypan blue exclusion. Concentration of viable cells was adjusted to 2×10^6 cells/ml in RPMI 1640 with 10 % fetal bovine serum.

Lectin-Induced Proliferation. One hundred microliters of the cell suspension was added to 96-well plates (Falcon, Oxnard) with 100 µl of various concentrations of the mitogens phytohemagglutinin (PHA ; Pharmacia Fine Chemicals, Bois D'Arcy), concanavalin A (Con A ; Pharmacia), soybean agglutinin (SBA : Pharmacia) and pokeweed mitogen (PWM ; Sigma Chemical Co, St Louis). Cells were incubated for 3 days at 37 °C, 7 % CO_2 and pulsed with 1 µCi methyl-^{3}H-thymidine (specific activity from 0.5 to 2.0 Ci/mM, Centre d'Énergie Atomique, Gif-sur-Yvette). Cultures were harvested 16 h later onto glass filter paper disks using a semi-automatic cell harvestor. Filter disks were dried and placed in scintillation vials with 4 ml of scintillant and counted in a beta scintillation counter.

In experiments where effects of antibody in the absence of complement were tested directly on proliferative activity, procedures were similar to above except 50 µl of cells, 50 µl of mitogen and 50 µl of control medium or PT 81 Moab in the form of a tissue culture supernatant were added to each well.

Statistical Methods. All experiments were analyzed as randomized, complete block designs (Steel and Torrie 1980) and Duncan's New Multiple Range Test (Duncan 1955) was used to evaluate the effects of different treatments. Standard errors of the mean were calculated from the pooled error mean square of the three experimental treatments.

Results

Production of Moab. Two weeks after fusion, 700 viable colonies were present in the HAT medium, and 99 (14 %) were positive for porcine PBL by a microcytotoxicity assay. Certain cell lines were cloned by limiting dilution and tested on PBL derived from at least 5 different pigs by flow microfluorimetry. The number of cells bound by antibody was fairly constant within each of the Moab tested. Moab were cloned that bound from 25 % to 100 % of PBL (table 1).

Table 3. – Surface markers on porcine PBL before and after treatment with Moab PT 81.

Surface marker	Cells (%)	Cells + Complement (%)	Cells + Complement + PT 81 (%)	SEM	Theoretical (1) (%)
Sheep rosettes	43[a]	43[a]	23[b]	2.5	0
Porcine rosettes	10[a]	11[a]	4[b]	1.5	0
Fcγ	6	6	8	0.8	10
C3b	4	3	3	1.1	5
Membrane Ig	15[a]	5[b]	10[c]	1.2	8
Null	42[a]	52[b]	67[c]	3.1	70

Each phenotypic marker was measured on PBL from the same five pigs. Averages within a row with different superscripts are different at $P < 0.01$. The SEM is pooled across each of the three treatments. Mean specific cytotoxicity with PT 81 was 37 %. Percentage null cells was calculated as 100 – (% rosettes with sheep erythrocytes + % membrane immunoglobulin positive).

1 : Theoretical percentage assuming that PT 81 lyses only cells that are positive for sheep and porcine rosettes.

Distribution of PT 81 Positive Cells. The Moab PT 81 was selected and characterized by flow microfluorimetry on cells from the thymus, blood and spleen that were depleted of phagocytic cells by treatment with carbonyl iron. As shown in table 2, background fluorescence with the fluoresceinated $F(ab')_2$ fragment ranged from 1 % to 8 %. After addition of PT 81, 66 % of the thymocytes, 47 % of the PBL and 38 % of the splenic lymphocytes were labeled.

Surface Markers. The average specific cytotoxicity with PT 81 on PBL from five pigs was 37 % (table 3), which compares favorably to the 39 % positive cells (47 % – 8 % background, table 2) detected on another group of five pigs tested by flow microfluorimetry. The three porcine PBL populations, consisting of the original population, cells treated with rabbit complement alone and cells treated with rabbit complement in the presence PT 81, were then tested using a variety of rosette assays to determine the type of cell recognized by PT 81 (table 3). Complement alone had little effect on any of the cell populations determined by rosette assays. This result indicated that there was little nonspecific loss of cells caused by complement. However, when the Moab PT 81 was added along with complement, there was a 47% reduction ($P < 0.001$) in the percentage of T cells. There was also a 64 % decrease ($P < 0.001$) in the percentage of cells within a specific T cell subset that binds porcine erythrocytes. This result showed that PT 81 identified a subset of T cells that recognized porcine erythrocytes more readily than PT 81 negative T cells.

Percentage of cells that carried the Fcγ and C3b receptors, as well as the percentage of cells with membrane Ig, were low in all treatment groups, so it was difficult to clearly evaluate the role of PT 81 on these lymphoid cell subsets. Cells with Fcγ receptors increased slightly (from 6 % to 8 %), as theoretically expected if PT 81 did not recognize these cells (table 3). There was no change in the percentage of cells with receptors for the third component of complement. Complement alone cause a reduction in the percentage of cells that stained positive for membrane Ig, from 15 % to 5 %. This effect may have been caused by an antibody in rabbit complement that specifically recognized porcine B cells, resulting in activation of complement and a resultant destruction of primarily B cells. However, after treatment with PT 81 plus complement, there was an increase ($P < 0.01$) from 5 % (complement alone) to 10 % (PT 81 plus complement) in percentage of cells that were positive for membrane Ig. This result suggested that PT 81 did not destroy B cells. Finally, as would be expected after removal of a major portion of T cells, there was a significant ($P < 0.05$) enrichment in the percentage of cells lacking surface markers that was very close to the calculated theoretical percentage of null cells (67 % *vs* 70 %).

Double Fluorescence Labelling. In order to directly determine whether PT 81 recognized an epitope on B cells, porcine splenocytes were double labelled with a rhodamine-labelled anti-Ig reagent and a fluoresceinated $F(ab')_2$ fragment that detected PT 81 (table 4). These studies clearly showed that PT 81 labelled cells that were distinctly different from cells that carried surface Ig. These data strengthen our earlier conclusion that PT 81 does not recognize porcine B cells.

Table 4. – Double labelling of porcine splenocytes with anti-Ig antibodies and PT 81.

	Positive Cells (%)		
Pig number	PT 81	Surface Ig	PT 81 and Surface Ig
1	36	26	1
2	21	26	0
3	22	23	0

Background fluorescence with fluoresceinated $F(ab')_2$ rabbit anti-mouse Ig was 4 %.

Lectin-Induced Proliferation. Cells from each of the three treatments were passed over Ficoll/Hypaque after various treatment procedures to remove dead cells and to restore all cell populations to a high viability. This approach yielded cells with viabilities of 96 % and 95 % on the original, complement and complement and PT 81 treatments. All cells were subsequently tested for their capability to proliferate *in vitro* in dose-response curves to plant lectins and incorporate a radiolabeled thymidine nucleoside tracer (table 5). Treatment with complement alone tended to enhance uptake of ^{3}H-thymidine, regardless of the mitogen that was used. More importantly, however, addition of PT 81 with the rabbit complement caused a significant reduction in the proliferation of cells with all doses of each mitogen tested. For PHA at stimulating dilutions of 1:10, 1:50 and 1:100, there was a corresponding reduction of 65 %, 85 % and 91 % when compared to complement-treated cells. Corresponding reductions caused by complement plus PT 81 for the other mitogens were : Con A at initial concentrations of 100, 50 and 10 μg/ml resulted in decreases of 85 %, 76 % and 78 % ; SBA at 50, 20 and 10 μg/ml yielded decreases of 80 %, 71 % and 72 % ; and PWM at 20, 10 and 1 μg/ml led to diminutions of 66 %, 77 % and 70 %. Thus, these results show a similar degree of reduction in proliferation regardless of the dose or mitogen that was used.

Lectin-Induced Proliferation with Moab in Absence of Complement. To determine whether the Moab PT 81 was blocking proliferation of cells by interfering with the capability of mitogens to bind to surface membrane components, lectin-induced assays were conducted in the presence of a 1/160 dilution of antibody in the total absence of complement. Three experiments were conducted, using all of the mitogen doses reported in table 5. The continual presence of PT 81 during *in vitro* cultivation of PBL with mitogens did not affect proliferative responses at any mitogen dose (data not shown).

Discussion

In this report, we have described the development of a panel of Moab that bind monomorphic determinants on porcine PBL. The antibodies recognized different percentages of cells, ranging from 25 % to 100 % of PBL. As described previously (Pescovitz *et al* 1984), these findings indicate that PBL in the porcine species are quite heterogeneous with respect to the antigens on their surface. Only one of these Moab was selected for characterization in this study because it detects a T cell subset as indicated by rosetting of sheep erythrocytes. It appears that this antibody recognizes a cell type that is necessary for proliferation induced by several different phytomitogens.

The cell type recognized by PT 81 is consistent with that of a T cell subset. There was a specific depletion of cells with a receptor for both sheep and porcine erythrocytes, which indicated that the major target cell for PT 81 is T cells. However, lysis with PT 81 plus complement depleted only 50 % of the T cells with receptors for sheep erythrocytes, which suggests that the Moab recognizes a T cell subset. Inducer/helper CD4 cells account for 45 % of porcine T cells, and suppressor/cytotoxic CD8 cells amount to approximately 50 % of porcine T cells (Pescovitz *et al* 1985). Thus, on the basis of the proportion of cells, Moabs CD4, CD8 and PT 81 define the same percentage of T cells.

After lysis with PT 81, there was an enrichment of cells bearing both surface Ig and Fcγ receptors, which suggests that the Moab does not bind B cells. This conclusion was confirmed by double labelling experiments. There was no enrichment in C3b positive cells, which may be partially explained by either : (1) the very low number of zymosan-complement rosettes observed among the three treatments or (2) the possibility that PT 81 also recognized a very small number of C3b positive cells. Finally, there was an enrichment in the number of null cells after lysis with PT 81 plus complement. If Moab PT 81 did not recognize any null

Table 5. – Proliferation of PBL stimulated with various phytomitogens in the original cell population, cells treated with rabbit complement alone and cells treated with complement and PT 81.

Mitogen	Counts per minute				Percent reduction from complement treated Cells (%)
	Cells	Cells + Complement	Cells + Complement + PT 81	SEM	
PHA					
1:10	48 099[a]	46 184[a]	16 389[b]	9 126	65
1:50	40 170[a]	54 275[a]	8 078[b]	11 776	85
1:100	32 070[a]	52 658[a]	4 859[b]	10 260	91
Con A (μg/ml)					
500	392	315	294	159	7
100	6 203[a]	14 240[b]	2 066[a]	3 800	85
50	38 494[a]	55 475[a]	13 197[b]	12 012	76
10	30 451[a]	57 914[a]	12 843[b]	9 555	78
SBA (μg/ml)					
50	28 547[a]	40 538[a]	8 080[b]	10 200	80
20	19 896[a]	29 376[a]	8 536[b]	6 536	71
10	13 493	8 749	2 478	6 253	72
PWM (μg/ml)					
20	22 324[a]	47 214[b]	16 037[a]	5 428	66
10	20 664[a]	34 905[a]	7 952[b]	5 288	77
1	15 362[a]	34 487[b]	10 337[a]	5 697	70

Five pigs were tested at each mitogen dose. Averages within a row with different superscripts are different at $P < 0.05$ or $P < 0.01$. In the absence of any mitogen (RPMI only), PBL, cells + complement and the cells + complement + PT 81 treatments incorporated 896, 1095 and 915 cpm, respectively. Percent specific cytotoxicity of PT 81 for the five pigs averaged 37 % ± 6.

cells, one would expect approximately 70 % null cells in this treatment. After lysis with PT 81 plus complement, 67 % null cells remained, which is consistent with this theoretical value.

It therefore appears that the decrease in mitogenic responses is due to the elimination of PT 81 positive responding cells. In the case where PT 81 positive and negative cells respond independently to lectins, there should be a 50 % reduction in the mitogenic response since treatment with PT 81 reduced the proportion of T cells by 50 %. However, we consistently observed around a 75 % reduction in lectin-induced proliferative responses, which suggests that an interaction between PT 81 positive and negative cell populations is needed for optimal mitogenic responses. However, such a conclusion needs further investigation since T cells among the PT 81 negative population do not represent more than 20 % of the T cells. It is possible that PT 81 removes CD8 cells and leaves CD4 cells since at least human CD4 cells respond to both PHA and Con A, whereas CD8 cells respond only to concanavalin A (Reinherz *et al* 1979 ; Reinherz and Schlossman 1980). Unfortunately, no direct comparison can be made in pigs since neither Pescovitz *et al* (1985) nor Jonjic and Koszinowski (1984) compared mitogenic responses to both PHA and Con A.

In summary, we have described the development of a panel of Moabs that recognizes varying populations of porcine PBL. One of these Moab recognizes a T cell subset that is necessary for proliferative responses to plant lectins, and none of these PT 81 positive cells are mature B cells. The Moab PT 81 and others in this panel should therefore be useful in studies aimed at understanding T cell differentiation and function in swine.

Note Added In Proof : Since the completion of this manuscript, labelling with PT 81 and PT8 has been compared using immunofluorescence techniques. The proportion of labelled cells, using either of the Moabs separately or in combination with each other, was similar for both PT 81 and PT8. This finding suggests that PT 81 and PT8 recognize the same lymphocyte subset.

Acknowledgements

This work was supported in part by grants to Keith W Kelley from the USDA-SEA (Nos. 58-519B1-1120 and 83-CRSR-2-2331), Moorman Manufacturing Company and the Washington and Illinois Agriculture Experiment Stations. The research was also supported by the Institut National de la Recherche Agronomique while Keith W Kelley was an invited research scientist on sabbatical leave from Washington State University. The authors wish to thank Charles Kissenger, Michel Olivier and Diane Roza for expert technical assistance.

Abstract

A panel of cytotoxic monoclonal antibodies (Moab) derived from mice immunized with porcine thymocytes has been developed which reacts with monomorphic determinants on porcine peripheral blood lymphocytes (PBL). These Moab recognized from 25 % to 100 % of PBL as tested by flow microfluorimetry One of the Moab (PT 81) that bound 39 % of PBL, 30 % of splenocytes and 65 % of thymocytes as determined by flow microfluorimetry was selected for initial characterization. PT 81 specifically lysed 37 % of Ficoll/Hypaque-prepared PBL depleted of monocytes by carbonyl iron ingestion. This antibody lysed 47 % of T cells (PBL that formed dextran-enhanced, sheep erythrocyte rosettes ; $P < 0.001$) and 64 % of a T cell subset (PBL that formed dextran-enhanced, porcine erythrocyte rosettes ; $P < 0.001$) when compared to cells treated with complement alone. Lysis of PBL with PT 81 plus complement caused a 100 % enrichment in the number of surface immunoglobulin positive cells ($P < 0.01$). The conclusion that PT 81 does not recognize B cells was further supported by double labelling experiments. Removal of PT 81 positive cells did not significantly affect the number of cells with C3b (zymosan-complement rosettes) or Fcγ (erythrocyte-antibody rosettes) receptors. PBL that were treated with PT 81 plus complement were passed over Ficoll/Hypaque (density 1.09) to remove dead cells. The PT 81-depleted lymphocyte population displayed only 20 %, 20 %, 26 % and 29 % of the proliferative responses of control cells treated with complement alone ($P < 0.01$) to several concentrations of the mitogens phytohemagglutinin, concanavalin A, soybean agglutinin and pokeweed mitogen, respectively. Incubation of the PT 81 Moab with PBL during cocultivation with the mitogens in the absence of complement did not affect proliferative responses. These results show that Moab PT 81 does not label B cells but recognizes an epitope present on only a subset of T cells, and that this T cell subset is essential for normal proliferative responses to plant lectins.

References

Buschman H, Pawlas S, 1980. A study of porcine lymphocyte populations. 2. Characterization of porcine lymphocyte populations. Vet Immunol Immunopathol 1:225-241

Davila DR, Franklin RA, Kleiss AJ, Kelley KW, 1987. A soluble 61-kDa protein is associated with inhibition of lectin-induced proliferation and IL-2 synthesis. Proc Soc Exp Biol Med 186:1-12

Duncan DB, 1955. Multiple range and multiple F tests. Biometrics 11:1-42

Goldstein P, Schirrmacher V, Rubin R, Wigzell H, 1973. Cytotoxic immune cells with specificity for defined soluble antigens. 2. Chasing the killing cells. Cell Immunol 9:211-225

Hammerberg C, Schurig GG, 1986. Characterization of monoclonal antibodies directed against swine leukocytes. Vet Immunol Immunopathol 11:107-121

Jonjic S, Koszinowski UH, 1984. Monoclonal antibodies reactive with swine lymphocytes. 1. Antibodies to membrane structures that define the cytolytic T lymphocyte subset in the swine. J Immunol 133:647-652

North J, 1980. Microcytotoxicity test. In Mishell BB, Shiigi SM (eds), Selected Methods in Cellular Immunology, 273-275, Freeman, San Francisco

Pescovitz MD, Lunney JK, Sachs DH, 1984. Preparation and characterization of monoclonal antibodies reactive with porcine PBL. J Immunol 133:368-375

Pescovitz MD, Lunney JK, Sachs DH, 1985. Murine anti-swine T4 and T8 monoclonal antibodies : Distribution and effects on proliferative and cytotoxic T cells. J Immunol 134:37-43

Reinherz E, Schlossman S, 1980. The differentiation and function of human T lymphocytes. Cell 19:821-827

Reinherz E, Kung PC, Goldstein G, Schlossman SF, 1979. Separation of functional subsets of human T cells by a monoclonal antibody. Proc Natl Acad Sci (USA) 76:4061-4065

Salmon H, 1978. Specificity of pig T lymphocyte antiserum : cytotoxicity and inhibiting properties onto E-rosette-forming lymphocytes. Ann Inst Pasteur (Immmunol) 129C:571-574

Salmon H, 1979. Surface markers of porcine lymphocytes and distribution in various lymphoid organs. Int Arch Allergy Appl Immunol 60:262-274

Salmon H, 1982. Characterization of pig lymphocyte receptors for allogenic and non-allogenic erythrocytes. 1. Apparent common identity of both receptors. Clin Exp Immunol 48:574-580

Steel RGD, Torrie JH, 1980. Principles and procedures of statistics. McGraw-Hill, New York, 633 p

Westly HJ, Kelley KW, 1984. Physiologic concentrations of cortisol suppress cell-mediated immune events in the domestic pig. Proc Soc Exp Biol Med 177:156-164