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MONOCLONAL ANTIBODIES REACTIVE WITH SWINE LYMPHOCYTES

I. Antibodies to Membrane Structures That Define the Cytolytic T Lymphocyte Subset in the Swine[1]

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A panel of monoclonal antibodies (mAb) with specificity for swine leukocytes was prepared by somatic cell hybridization with the use of spleen cells from mice immunized with swine thymocytes. The reactivity of two mAb (295/33 and 122/28), which both immunoprecipitated from the surface of swine leukocytes an antigen termed S-L2 with an apparent m.w. of 33 to 35 kilodaltons under reducing and 65 to 70 kilodaltons under nonreducing conditions, was investigated in detail. These mAb were reactive in indirect immunofluorescence with 50 to 60% of thymocytes, 35% of peripheral blood lymphocytes, and 55% of E rosette-positive cells; they were nonreactive with bone marrow cells, Ig^+ B cells, nonrosetting lymphocytes, granulocytes, and monocytes. In functional studies, the elimination of S-L2$^+$ cells partially reduced the proliferative response to concanavalin A and pokeweed mitogen but not to *Staphylococcus aureus* and *lipopolysaccharide*. The S-L2$^-$ subset proliferated well to alloantigens. Both cytolytic T effector cells and precursor cells carried the antigen S-L2 and could be depleted from heterogeneous cell populations by both antibodies in the presence of complement. These data suggest that the mAb 295/33 and 122/28 recognize a specific polypeptide present on the surface of swine cytolytic T cells. These antibodies will be useful in studies on the swine immune system.

Although several methods have been used for the morphologic and functional differentiation of T and B lymphocytes and their subpopulations (1, 2), monoclonal antibodies (mAb)[4] represent the most appropriate tools to identify cell surface antigens and to study the functional properties of cells expressing them. So far mAb have been used to identify lymphocyte subpopulations in mouse, human, rat, hamster, and dog (3–8).

The need to investigate and to understand the function of the immune system in domestic animals with particular emphasis on their role in the defense against various pathogens is obvious. For these and also for experimental reasons, the immune system of the swine has been studied and inbred lines have been established (9–13). Unfortunately, the characterization of T lymphocytes is based so far mainly on the finding that swine T lymphocytes have receptors for sheep red blood cells (SRBC) (13, 14); however, not all T lymphocytes seem to form E rosettes and the efficiency of cell separation with this technique is variable. Therefore, the swine lymphocyte populations involved in the regulation and function of the immune response are still poorly defined.

In the present study we describe the production of mAb that bind to swine T lymphocytes and the characterizationof these mAb with regard to distribution and some functional properties. Two antibodies (mAb 295/33 and 122/28), which identify a subpopulation of E^+ T cells, were analyzed in more detail. The results show that these mAb recognize a molecule with an apparent m.w. of about 33 to 35 kilodaltons. These molecules are present on cytolytic T lymphocytes (CTL) and their precursor cells and, as tested so far, are absent on B cells and other T cell subsets. These mAb will be useful for the identification, selection, and further characterization of this T cell subset on the swine.

MATERIALS AND METHODS

Animals. BALB/c mice were bred at our own facility and were used at 8 wk of age. Outbred swine were obtained from various sources.

Production of mAb. BALB/c mice were immunized three times with swine thymocytes. The mice were first injected subcutaneously with 5×10^6 thymocytes in complete Freund's adjuvant (Sigma, St. Louis, MO) and again 2 wk later i.p. with the same cell number suspended in incomplete adjuvant. Four days before fusion, the animals were boostered with an i.p. injection of 5×10^6 thymocytes. Spleen cells from immunized mice were fused with the SP2/0-Ag14 myeloma line in the presence of polyethylene glycol. After fusion, cells were washed and resuspended in HAT[4] selective medium/RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% FCS, 100 U/ml penicillin-G, 0.1 g/liter streptomycin, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES buffer, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine. Cells were cultured in 24-well plastic plates (Costar, Cambridge, MA) at 37°C with a humidified 5% CO_2 atmosphere. Beginning on day 5, the plates were inspected daily for cell growth and supernatants from growing hybridomas were screened by the cell-ELISA[4] and the C-mediated cytotoxicity assay with ^{51}Cr-labeled thymocytes, lymphocytes, or granulocytes used as targets. Hybridomas from wells scored positive were cloned by limiting dilution in the presence of BALB/c peritoneal cells used as feeder cells. They were then grown in bulk tissue

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[4] Abbreviations used: CTL, cytolytic T lymphocytes; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; HAT, hypoxanthine, aminopterin, thymidine; mAb, monoclonal antibody; PWM, pokeweed mitogen; Staph-A, *Staphylococcus aureus*.

culture in RPMI 1640 supplemented with 10% FCS or as ascites in pristane (2, 6, 10, 14-tetramethyl pentadecane; Aldrich, Milwaukee, WI)-treated mice. Characterization of mAb was carried out by Ouchterlony radial immunodiffusion analysis with subclass-specific antisera (Nordic, Tilburg, The Netherlands and Bionetics, Charleston, SC).

Cell separation procedures. Peripheral blood leukocytes (PBL) were obtained by Lymphoprep (Nyegaard, Oslo, Norway) gradient centrifugation of fresh heparinized blood. T lymphocytes were separated from other mononuclear cells by E rosetting with 5% SRBC (16). The cell suspension was incubated at 37°C for 10 min, centrifuged at 200 × G for 10 min, and incubated overnight at 4°C. The resuspended sediment of the rosetting mixture was layered on Lymphoprep and after centrifugation T- or B-enriched populations were recovered from the E^+ sediment and the E^- interphase. By indirect immunofluorescent staining, 40 to 60% of E^- cells were found to be surface Ig^+ B lymphocytes, but only a few surface Ig^+ cells were found in the E^+ fraction. Ig^+ cells were identified by staining with polyvalent fluorescein isothiocyanate (FITC)-conjugated rabbit anti-pig Ig (Miles-Yeda, Rehovoth, Israel). Granulocyte-enriched cell populations were obtained from the lymphoprep centrifugation sediment after lysis of erythrocytes with Gay's solution.

Cell-ELISA. With some modification, established methods were used (16). Briefly, 50 μl of cell suspension were distributed in U-bottomed microtiter plates and fixed with 0.25% glutaraldehyde. After incubation for 2 hr at 37°C or overnight at 4°C, supernatants were removed and PBS with 2% BSA was added for 2 hr. PBS/BSA was removed and 50 μl of the hybridoma supernatant were added for 1 hr at room temperature. The plates were washed three times in PBS-Tween 20 (Sigma) (Tween 20 concentration 0.05%) and 50 μl of goat anti-mouse Ig $F(ab')_2$ peroxidase-conjugated antibodies (TAGO, Burlingame, CA) were added for 1 hr. The plates were washed again with PBS-Tween 20, and 50 μl of substrate (ortho-phenylenediamine + H_2O_2) were added for 1 hr at room temperature. The reaction was stopped by adding 25 μl of 1 M H_2SO_4 and then the optical density was measured.

C-dependent cytotoxicity and depletion of lymphocytes. Swine thymocytes, lymphocytes, granulocytes, and Con A blast cells were labeled with ^{51}Cr (Amersham, Buchler, Braunschweig, FRG). Dead cells were removed by Lymphoprep gradient centrifugation. After incubation of 5 to 10 × 10^3 ^{51}Cr-labeled cells in 50 μl with 50 μl of hybridoma supernatant or ascites fluid for 30 min at 4°C, rabbit C (100 μl) was added for 1 hr at 37°C. Then 100 μl of supernatant were removed for counting. The spontaneous release was obtained from target cells incubated with medium and C alone.

The cytotoxic titer was defined as the dilution resulting in 50% of the maximal specific lysis observed. The cytotoxic titer of the supernatant-derived mAb used in this study on the various target cells ranged between 1/2000 and 1/3000. For depletion studies, cells were subjected to two cycles of treatment with antibody (final dilution 1/20) and C if not stated otherwise. The efficiency of the treatment was evaluated by counting residual viable cells.

Immunofluorescence staining. Single cell suspensions were incubated with saturating concentrations and dilutions of mAb in RPMI 1640 supplemented with 2% FCS and 0.01% NaN_3. After 30 min at 4°C, the cells were washed three times, and FITC-conjugated goat anti-mouse IgG (H and L chains) (Cappel, Cochranville, PA) was added and incubated for another 30 min at 4°C. After washing, the cells were resuspended in Hanks'-BSS with 0.2% BSA, 0.01% NaN_3 and 20 mM EDTA, and were kept on ice and used for microscopic inspection or for flow microfluorometry.

Flow microfluorometry. The single parameter flow microfluorometry measurements were carried out on a fluorescence-activated cell sorter (FACS IV; Becton Dickinson FACS Systems, Sunnyvale, CA). A 2-W argon ion laser (300 mW, Spectra Physics model 164-06) was used for excitation at 488 nm. Emission was measured through a 520-nm band pass filter (maximum transmission 84%, FWHM 30 nm and a 523-nm band pass filter (maximum transmission 63%, FWHM 12 nm). The fluorescence signal was triggered on particles of size greater than platelets. The dual scatter gate was set to exclude most dead cells. The logarithmically amplified fluorescence signal is represented on a 255-channel scale covering about 5 decades. At least 1 × 10^4 live cells were analyzed in each experiment and the histograms were area corrected to the determination of 10^4 cells. Supernatants derived from irrelevant hybridoma cell lines resulted in background levels of fluorescence.

Lymphocyte proliferation assays. PBL were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 5 × 10^{-5} M 2-mercaptoethanol, 10 mM HEPES, and antibiotics in 0.2-ml volumes in round-bottomed microtiter plates (Greiner, Nürtingen, FRG) at 37°C in a 5% CO_2 atmosphere. The mitogens Con A (10 μg/ml) (Sigma), pokeweed mitogen (PWM) (5 μg/ml) (Serva, Heidelberg, FRG), inactivated Staph-A^4 (250 μg/ml) (Pansorbin, Calbiochem; Gießen, FRG), and LPS (125 to 1000 μg/ml) (Sigma) were present during a 3-day period. For alloantigen-induced proliferation, the mixed leukocyte reaction (MLR) was set up by co-culturing 2.5 × 10^5 responding PBL with 2 to 3 × 10^5 irradiated (2000 rad) or mitomycin C (Roth, Karlsruhe, FRG)-treated stimulating PBL in round-bottomed microtiter plates (Greiner) for 5 days (12). The proliferative response was determined by the incorporation of [^{3}H]thymidine (Amersham) that was added in a concentration of 1 μCi/well 18 hr before the cultures were harvested on a Skatron multiharvester (Lierbyen, Norway). [^{3}H] Thymidine uptake was quantified by liquid scintillation counting.

Cell-mediated lysis. Cells from 7-day MLR were used as a source of CTL. ^{51}Cr-labeled Con A blasts were used as targets. To test the influence of mAb and C on CTL effector function, cells were treated after MLR expansion. CTL precursors were treated before MLR with antibody plus C and the activity of the remaining cells was tested in cell-mediated lysis assay at day 7. The 3- to 5-hr assay was performed as described (17, 18). The percentage of specific cytolytic activity was calculated as follows:

$$\text{Percent specific cytotoxicity} = \frac{\text{cpm experimental release} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}}$$

Immunoprecipitation analysis. The lactoperoxidase method (see Reference 24) was used to label cell surface molecules. PBL were labeled with 1 mCi ^{125}I (Amersham) by incubation at room temperature for 10 min in 1 ml PBS containing 40 μg/ml lactoperoxidase (Sigma) and 10^{-5} M sodium iodide. To initiate the iodination, 10 μl of freshly prepared 10^{-2} M H_2O_2 were added. At 5-min intervals, 5 μl of additional H_2O_2 were added until a total of six additions was reached. After washing, the cells were lysed by sonication in extraction buffer (10 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 1% sodium deoxycholate, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), 2 mM L-methionine, 1 mg/ml ovalbumin, and 1 mM phenylmethylsulfonyl fluoride) and the lysates were centrifuged at 150,000 × G. Supernatant aliquots of 90 μl were incubated with 100 μl antibody dilution (60 min at 20°C and 60 min at 4°C). The immune complexes were precipitated by 0.05 ml Staph-A. The precipitates were analyzed in 10% SDS polyacrylamide slab gels (19). Gels were processed for fluorography with En^{3}Hance (New England Nuclear) and bands were visualized with Kodak X-Omat R films.

RESULTS

Initial screening. Spleen cells from BALB/c mice immunized with swine thymocytes were fused with SP2/O-Ag14 cells and cultured in HAT medium. Of 240 wells, more than 200 contained vigorously growing cells 3 wk after fusion. The majority of the supernatants were found positive in ELISA on swine thymocytes and supernatants from several wells lysed swine thymocytes and peripheral lymphocytes in the presence of C. Hybrid cells producing cytolytic antibodies were cloned and subcloned by limiting dilution and several clones 295/33, 122/28, 99/58, and 201/1 were selected for further study. The classification of these antibodies is given in Table I.

Reactivity with lymphoid cells. To analyze the cellular distribution of antigens identified by the mAb, single cell suspensions from bone marrow, thymus, spleen, and PBL were prepared and cells positive after indirect immunofluorescence were counted. Table I gives the cumulative data from several experiments showing the distribution patterns of the mAb 295/33, 122/28, 99/58, and 201/1. The mAb 99/58 stained about 52% of bone marrow and spleen cells, and a high percentage of thymocytes, peripheral lymphocytes, and granulocytes. The mAb 201/1 revealed a more diffuse reaction pattern and stained a high percentage of thymocytes, spleen cells, and peripheral blood T and B cells but did not react with granulocytes. The mAb 295/33 and 122/28 revealed an almost identical distribution pattern. These mAb did not stain bone marrow cells, reacted with about 50% of thymus cells, up to 25% of spleen cells, and about one-third of PBL. The granulocyte-enriched fraction obtained after

TABLE 1
Distribution of antigen-positive cells

mAb	Mean % Positive Cells[a]							
	Bone marrow	Thymus	Spleen	PBL[b]	E^+ cells	E^- cells	Con A blasts	Staph-A blasts
295/33, IgG2a, κ	<5	50–60	25	35–41	53	<4	58	<3
122/28, IgG2a, κ	<2	50–60	7–20	35	58	<5	53	<2
99/58, IgM, κ	52	72–92	47	70–85	87–91	20–52	86–95	<2
201/1, IgM, κ	<2	83	42	86–95	62–93	60	85	65

[a] Mean percent of cells stained by supernatant-derived mAb in indirect immunofluorescence. At least 200 cells were counted. Percentage of cells stained by second-layer antibody alone, about 3 to 5%, was not subtracted. Data are of three independent experiments with different swine used as cell donors. Numbers of 5% and smaller values are in the range of the arbitrary negative cutoff.

[b] Percentage of PBL reactive with antibody specific for swine IG was 20 to 27%.

fractionation of cells by Lymphoprep sedimentation was also unreactive with the latter two mAb (data not shown). The fact that these two mAb reacted only with a fraction of cells was indicative of their reactivity with a lymphocyte subset of thymic and post-thymic cells. To investigate the antigen distribution of cells enriched for T or B cells, PBL were fractionated into the E rosetting and nonrosetting subsets. In addition the lymphoblast fractions were prepared after stimulation with the T cell mitogen Con A or Staph-A as a mitogen for B cells (20).

With the exception of mAb 201/1, none of the mAb did react with Staph-A lymphoblasts. Whereas mAb 201/1 and 99/58 bound to most Con A lymphoblasts, mAb 295/33 and 122/28 stained only about 50% of the Con A lymphoblasts, which indicated a predominant reactivity with T cells. Immunofluorescence binding studies with E^+ and E^- cells supported these findings. The mAb 295/33 and 122/28 reacted only with about 50% of the E^+ cells but not with E^- cells, whereas mAb 99/58 reacted with practically all E^+ cells and in addition with variable numbers of E^- cells. These results led to the conclusion that all three mAb—295/33, 122/28 and 99/53—react with T cells but not B cells and that mAb 295/33 and 122/28 are apparently reactive only with a T cell subset. The FACS analysis of the mAb 295/33 and 122/28 confirmed these observations. Figure 1*A* shows a representative fluorescence profile after reacting peripheral lymphocyte suspensions with the mAb and FITC-labeled goat anti-mouse Ig (H and L chains). About 30 to 40% of peripheral lymphocytes were stained by both mAb with low to medium intensity. In addition, the FACS profiles, as judged by the intensity of fluorescence, suggested the presence of two populations. The analysis of the monocyte-enriched fraction of peripheral leukocytes revealed few fluorescent cells (Fig. 1*B*). Thus, only a subset of the E^+ lymphocyte population is reactive with mAb 295/33 and 122/28, whereas B cells and monocytes remain unstained.

Furthermore, the nonreactivity with B cells was also observed when the cell populations were first treated with mAb 295/33 and 122/28 plus C and then stained with an FITC-labeled antibody to swine Ig. This procedure resulted in about 30% trypan blue-stained cells and a clear relative increase of cells carrying swine Ig on the surface (data not shown).

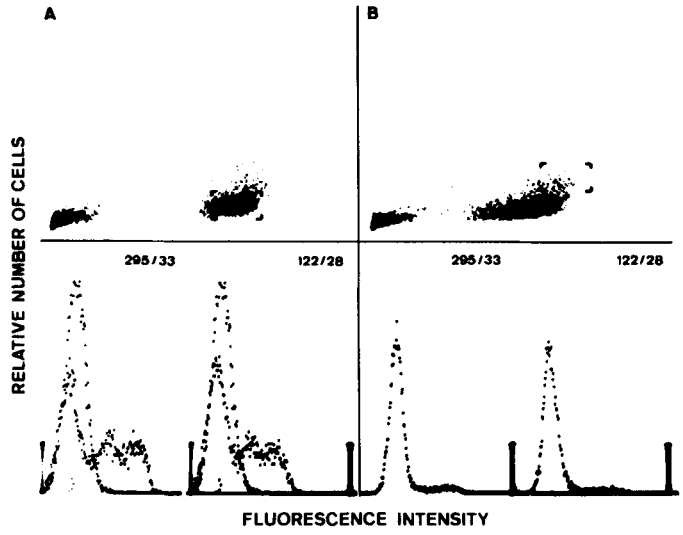

Figure 1. Fluorescence histograms from FACS analysis of peripheral swine blood mononuclear cells labeled with mAb. Upper panel: *A*; light scatter gating for lymphocytes, excluding contaminating erythrocytes and dead cells. *B*, light scatter gating for the predominantly monocyte (high scatter) region. Lower panel: comparative reactivity of mAb (ascites dilution 1/500) 295/33 and 122/28 against lymphocytes and monocytes (first step) followed by FITC goat anti-mouse Ig (H and L chains) (second step). All samples show a similar pattern of reactivity above control background (second step only. Note that in the analysis of the monocyte-enriched fraction the presentation of the background is omitted). Results are expressed on a log fluorescence scale with 10^4 cells analyzed.

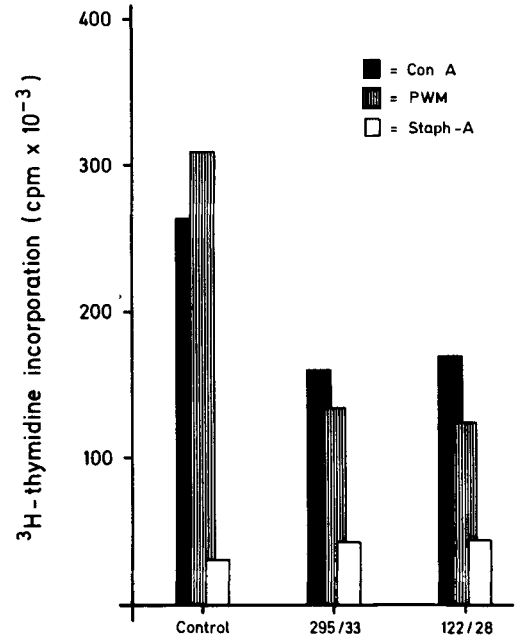

Figure 2. Effect of mAb on the proliferative response to mitogens. Peripheral swine blood lymphocytes (2.5×10^5) were treated twice with the indicated mAb derived from hybridoma supernatants plus C or with C only, and then were incubated without correction of cell numbers with 10 μg/ml Con A, 5 μg/ml PWM, or 250 μg/ml Staph-A. Results represent mean of six replicate cultures. Stimulation indices (ratio mitogen stimulated:control cultures) were: 81 for Con A, 90 for PWM, and 10 for Staph-A.

Functional studies. The distribution studies revealed that the cells identified by the two mAb belong exclusively to a T cell subset. Functional studies were performed to see whether this subset correlates with specific functional activities.

Inhibition of the proliferative response to mitogens and alloantigens. Pretreatment of lymphocytes with 295/33 and 122/28 plus C reduced the subsequent pro-

liferative response to the T cell-activating mitogens Con A and PWM by about 40 to 50% (Fig. 2). The percentage of reduction of the proliferative response to these mitogens was similar in repeated experiments. The response to the T and B cell mitogen Staph-A and the B cell mitogen LPS was poor in comparison with T cell mitogens, but was not reduced. A response to LPS was not seen at concentrations lower than 125 μg/ml (Fig. 2, Table II). These data confirmed again the T cell specificity of the mAb, but did not help to discriminate between functionally distinct T cell subpopulations.

Pretreatment of lymphocytes before the mixed lymphocyte culture with autologous or allogeneic irradiated lymphocytes gave more indicative results with regard to the T cell subset concerned (Table III). Both mAb plus C lysed about 30% of cells as determined by trypan blue exclusion. This treatment, however, did not reduce either the autologous or the allogeneic MLR whereas treatment with mAb 201/1 and C strongly diminished the proliferative response. This finding led to the conclusion that the helper T cell subset, which is required for the proliferative response to alloantigens *in vitro*, is not affected by the mAb 295/33 and 122/28.

TABLE II

The mAb 295/33 and 122/28 do not affect the proliferative response to LPS[a]

LPS Concentration (μg/ml)	Treatment of Cells		
	C	295/33 + C	122/28 + C
—	1319 ± 95	1385 ± 58	1546 ± 56
125	3276 ± 276	3986 ± 217	4608 ± 35
250	3903 ± 128	4776 ± 176	5732 ± 773
500	3146 ± 190	4306 ± 677	5201 ± 449
1000	3946 ± 124	5315 ± 138	5306 ± 80

[a] Responder lymphocytes, after treatment with supernatant-derived mAb and C, were incubated with different concentrations of LPS. Cell numbers were not corrected to equal the cell counts of C-treated controls. Thymidine incorporation was determined after 72 hr of culture. Results represent the mean of three replicate cultures in cpm ± SEM.

TABLE III

Effect of antibodies on T cell proliferation in MLR[a]

Responder Cell Treatment	Proliferation Induced by	
	Autologous lymphocytes	Allogeneic lymphocytes
C alone	1,770 ± 280	19,935 ± 1591
295/33 + C	1,695 ± 64	22,591 ± 1697
122/28 + C	1,708 ± 150	22,648 ± 2748
201/1 + C	3,015 ± 532	1,961 ± 98[b]

[a] Responder lymphocytes (2.5×10^5) were cultured for 4 days together with 3×10^5 irradiated (2000 rad) allogeneic or autologous lymphocytes. mAb from hybridoma supernatants were used for cell depletion. Cell numbers were not corrected after treatment to match the cell counts of C-treated controls. Results represent the mean of six replicate cultures in cpm ± SEM.

[b] Inhibition significant at $p < 0.001$ by Welch's version of the *t*-test.

Reactivity with CTL and CTL precursors. Because the mAb 295/33 and 122/28 did not inhibit the proliferative activity in the allogeneic MLR, we tested to determine whether these antibodies could subdivide helper and cytolytic T cells. Figure 3*A* shows the results of a representative experiment designed to assay the ability of the mAb to lyse cytolytic effector cells. Responder cells were cultivated for 7 days either in the presence of syngeneic or allogeneic irradiated stimulator cells. The effector cells generated were analyzed in a cell-mediated lysis assay against ^{51}Cr-labeled syngeneic or allogeneic lymphoblast targets at various effector to target ratios (only a ratio of 100:1 is shown in Fig. 3*A*). All effector cells with cytolytic activity against lymphoblast target cells were susceptible to treatment with both mAb and C. Thus, mature CTL express determinants recognized by mAb 295/33 and 122/28. To determine whether CTL precursor cells already express the antigen(s) recognized by the two mAb, native swine lymphocytes were treated with mAb plus C. The remaining cells were used as responder cells and stimulated with irradiated syngeneic and allogeneic cells. After 7 days of culture, the cells were recovered and tested for cytolytic activity. Pretreatment with either mAb 295/33 or 122/28 completely abolished the ability of the responder cells to mount a detectable cytolytic response (Fig. 3*B*) whereas the capacity to generate a proliferative response (data not shown) again remained unaffected. Thus, CTL precursors as well as mature CTL express the antigen(s) recognized by mAb 295/33 and 122/28.

Analysis of the antigens detected by mAb 295/33 and 122/28. Immunoprecipitation experiments were performed to determine the m.w. of the antigens found on swine CTL. Data are shown only for mAb 295/33, but mAb 122/28 gave identical results (Fig. 4). PBL were surface-labeled with ^{125}iodine by the lactoperoxidase

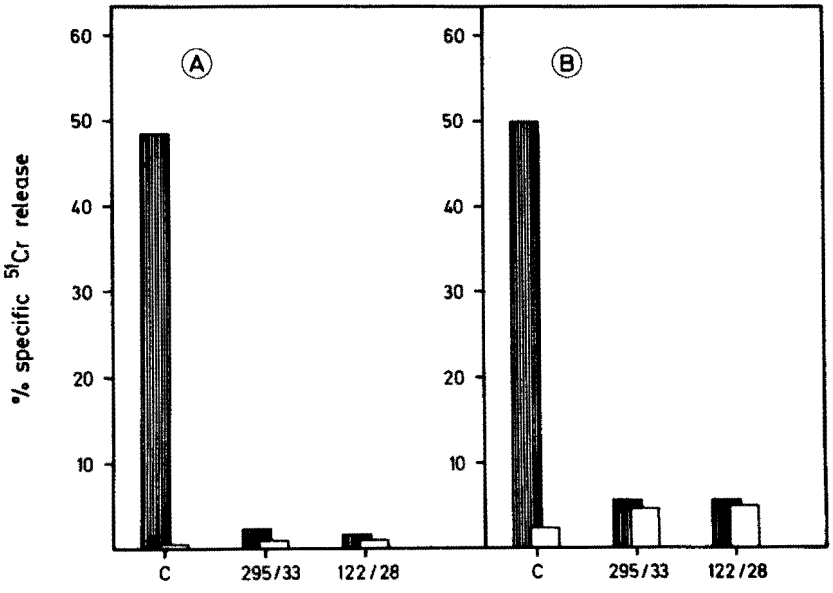

Figure 3. Treatment of CTL and CTL precursors with mAb and C. *A*, treatment of effector CTL. MLR cultures were set up with 3×10^6/ml monocyte-depleted, Lymphoprep-fractionated swine PBL responder cells and 3×10^6 irradiated, allogeneic stimulating cells. Seven days later, cultures were harvested and treated twice with the indicated mAb derived from hybridoma supernatants and C. Viable cells were recovered and incubated with ^{51}Cr-labeled autologous (*open columns*) or allogeneic Con A blasts (*hatched columns*) at effector to target ratio of 100:1. Percent specific lysis was measured after 3 hr of incubation. Spontaneous ^{51}Cr release was 12% from autologous and 12% from allogeneic target cells. Data represent mean cytolytic activity of six replicate cultures. *B*, treatment of CTL precursors. Preparation of responder and stimulator cells as in *A*. First 2×10^5 responder cells were seeded in six replicates into microtiter wells, treated twice with mAb plus C, and without correction of responder cell numbers to the cell counts of the C-treated controls, 2×10^5 stimulator cells were added. Cytolytic activity of effector cells derived from the six replicate cultures was individually tested at day 7 by adding 10^3 ^{51}Cr-labeled target cells in a 5-hr assay. Spontaneous release of ^{51}Cr was 14% from autologous and 15% from allogeneic target cells. In this experiment, effector to target ratio of C-treated controls corresponds on average to 100:1.

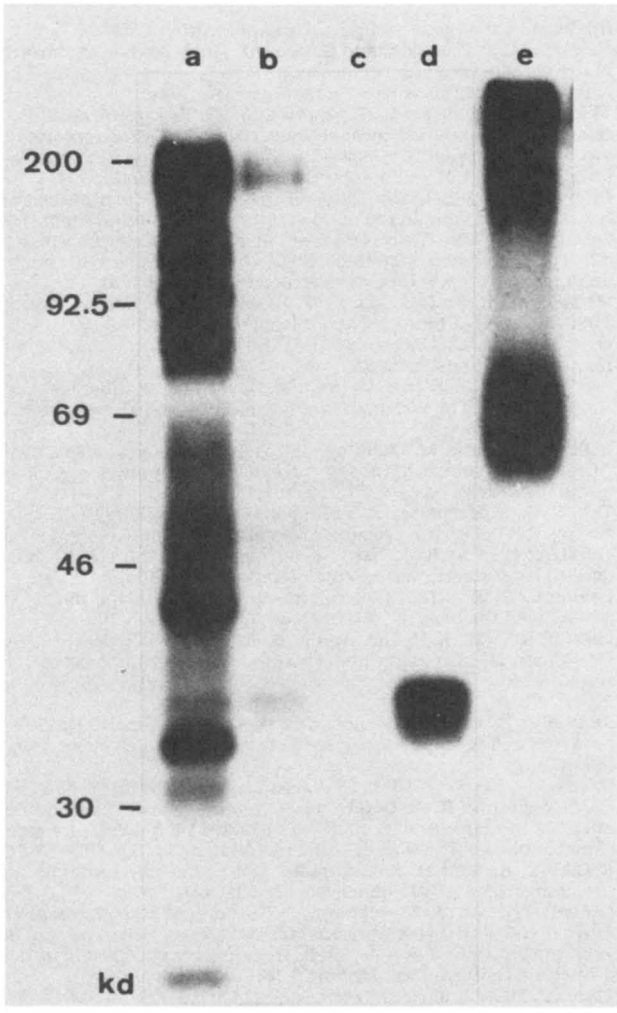

Figure 4. Immunoprecipitation of S-L2 antigen. Five to 15% SDS-PAGE of ^{125}I-labeled PBL. Track a, direct fractionation of 3.5×10^4 cell equivalents of lysate. Lysate equivalent to 1.25×10^6 cells was immunoprecipitated and separated under reducing conditions in track b with murine antiserum to swine thymocytes, in track c with negative control mAb, in track d with mAb 295/33 (derived from ascites fluid 1/100), and in track e also with mAb 295/33 but separated under nonreducing conditions. Molecular weight markers included: myosin (200 kilodaltons (kd)), phosphorylase b (92.5 kd), BSA (69 kd), ovalbumin (46 kd), and carbonic anhydrase (30 kd). Tracks a–d were exposed for 24 hr and track e for 7 days.

method, extracted, and incubated with mAb, and the precipitate was analyzed by SDS-PAGE and autoradiography. Under reducing conditions, the precipitated polypeptide migrated as a broad band of 33 to 35 kilodaltons. Under nonreducing conditions, peptides corresponding to a size of 65 to 70 kilodaltons as well as peptides of 190 kilodaltons and even higher m.w. were precipitated, which indicates that the precipitated peptide is composed of two disulfide-bonded subunits and forms dimeric and multimeric structures. Although both mAb apparently immunoprecipitated the same polypeptide, it remains to be determined whether they detect identical or distinct antigenic epitopes carried by the same molecule. The antigen found on swine CTL and defined by mAb 295/33 and 122/28 was termed S-L2.

DISCUSSION

In this study, we analyzed the production and characterization of mAb directed to a cell surface antigen present on a fraction of swine PBL. The antibodies resulted from a fusion between SP 2/0 myeloma cells and spleen lymphocytes from mice immunized to pig thymocytes. The two mAb 295/33 and 122/28 were characterized in detail. We present evidence here that: I) mAb 295/33 and 122/28 stain about 30% of peripheral lymphocytes, which are all E^+ rosette-forming cells; II) the antigen, termed S-L2, detected by these mAb is present on lymphoblasts generated by T cell mitogens and is absent on Ig^+ cells, B cell blasts, granulocytes, and monocytes; III) the antigen S-L2 is precipitated by both mAb from ^{125}I surface-labeled lymphocytes as a structure with an electrophoretic mobility as determined by SDS-PAGE analysis corresponding to an apparent m.w. of 33 to 35 kilodaltons under reducing conditions and 65 to 70 kilodaltons under nonreducing conditions; IV) the elimination of S-$L2^+$ cells does not interfere with the proliferative response to alloantigens; V) the elimination of S-$L2^+$ cells abrogates the generation of CTL *in vitro* and the effector function on *in vitro* generated CTL. Thus, in the swine, the molecule S-L2 detected by the mAb 295/33 and 122/28 on the swine CTL appears to be the structural homologue to the antigen detected on human T cells by the Leu-2a/T8 antibodies (21, 22), on mouse T cells by the anti-Lyt-2 (3), and on rat T cells by the MRC OX-8 antibody (6).

This conclusion is derived from several lines of evidence. First, in all four species, the tissue distribution reveals a high number of antigen-positive cells in the thymus whereas only one-third of peripheral T lymphocytes react with the specific antibody. Secondly, the 33 to 35 and 65 to 70 kilodalton polypeptides immunoprecipitated from swine cells under reducing and nonreducing conditions correspond with regard to m.w. to antigens of similar molecular size (22) found on human T cells (22, 23), murine T cells (24), and rat T cells (25). Thirdly, in all four species, this antigen identifies the CTL subset. There are some species differences with regard to distribution of the antigens. In man, OKT8 binds to granular leukocytes (26) and in the rat MRC OX-8 is also present on natural killer cells (25). The distribution data argue against the presence of S-L2 on granulocytes; however, the presence of this marker on separate swine subpopulations with cytolytic functions needs to be investigated. Natural killer cells are not yet clearly defined in the swine, and for this purpose the *in vitro* cultivation and assay systems for swine lymphocyte subpopulations must be improved. Improvement of the *in vitro* culture conditions will also be crucial to investigate whether the T suppressor cell subset is also defined by this antigen as it is the case in the other three species.

So far it is not clear whether S-L2 represents also the functional homologue to the molecule present on the suppressor/cytolytic T cell subset of the other species (22–25). Although the addition of the mAb to the MLR and to the cell-mediated lysis assay did not result in the inhibition of effector cell generation or function (unpublished observations), these data may be the result of imperfect *in vitro* culture conditions and must be considered preliminary. Experiments to investigate these open questions are under way.

Regardless of the function of the surface molecule recognized, the mAb to S-L2 promise to be useful reagents. The mAb can be used to separate a T cell subset from lymphocyte populations without affecting the lym-

phoproliferative response to alloantigens. Swine cytolytic T cells can be identified, depleted, or positively selected by various methods and the investigation of their role during physiologic and pathophysiologic conditions is open for further studies.

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