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MONOCLONAL ANTIBODIES REACTIVE WITH SWINE LYMPHOCYTES

II. Detection of an Antigen on Resting T Cells Down-Regulated After Activation[1]

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The expression of an antigen on porcine T lymphocytes detected by murine monoclonal antibody (mAb) 8/1 was investigated by functional studies and dual-parameter immunofluorescence. mAb 8/1 reacts with >95% of thymocytes and in peripheral blood with all T lymphocytes and with cells of the monocyte/macrophage lineage, but not with B cells, erythrocytes, and platelets. Pretreatment of peripheral blood lymphocytes with mAb 8/1 plus complement abrogated the proliferative response in vitro to mitogen, soluble antigen, and MHC determinants. Dual-parameter immunofluorescence revealed that resting porcine T8$^+$ as well as T4$^+$ lymphocytes express the 8/1 antigen, whereas after in vitro activation, cell surface expression of the antigen was low or absent in both T cell subsets. Thus, the 8/1 antigen represents a marker that discriminates between resting and activated T lymphocytes. Distribution and functional criteria indicate that 8/1 represents a novel marker not described before for any other mammalian species.

T lymphocytes express various cell surface markers that distinguish lymphocyte subpopulations with regard to maturation and function. Monoclonal antibodies represent the primary tools for the identification and characterization of these cell membrane molecules. Several lymphocyte antigens appear to be structurally conserved among mammalian species. The structural analogs to the human T4 (CD4) and T8 (CD8) antigens have been found in mouse, rat, sheep, and swine (1–7). The porcine T8 antigen detected by the monoclonal antibodies (mAb)[5] 295/33, 122/28 (4), and 76-2-11 (αPT8) (5, 6) is characterized as a protein of 30,000 to 35,000 m.w. under reducing conditions (4, 5). As has been shown by functional assays, these mAb define the cytolytic/suppressor subset of T lymphocytes ($T_{C/S}$). mAb 74-12-4 (αPT4) precipitates a 55,000 m.w. protein and is supposed to identify the porcine T lymphocyte helper/inducer subset ($T_{H/I}$), although the helper function of porcine T4$^+$ cells has not been demonstrated as yet (5, 6).

In addition to markers which characterize functional T cell subsets, lymphocytes exhibit significant changes in the expression of cell surface antigens during maturation and differentiation (8, 9). Furthermore, cell surface antigens exist that are expressed only on activated but not on resting T lymphocytes (10, 11) and, vice versa, antigens that are expressed on resting lymphocytes can be down-regulated during activation (12, 13). Both types of antigens define stages of T cell activation.

This report describes experiments in which the expression of a novel antigen on porcine T4$^+$ and T8$^+$ lymphocytes was studied with mAb 8/1. The results of flow cytometry and functional analysis show that this antibody is specific for a surface antigen present on immature thymic T cell precursors and resting post-thymic T cells, but is lost from porcine $T_{C/S}$ as well as $T_{H/I}$ cells after in vitro stimulation with antigen or mitogen. This mAb identifies a cell surface antigen of T cells which has not been described for any other mammalian species.

MATERIALS AND METHODS

Animals. BALB/c mice were bred at our own facility and were used at 8 wk of age. Porcine lymphoid tissues and blood were derived from randomly selected outbred animals housed at our facility or were obtained from the abattoir.

Monoclonal antibodies (mAb). The mAb 295/33 (αT8, IgG2a,κ), 122/28 (αT8, IgG2a,κ), 8/1 (IgG2b,κ), and 305/44 (IgG2b,κ) were derived from BALB/c mice immunized with porcine thymocytes. Spleen cells were fused with SP2/0, and resulting hybridomas were assayed as described (4). mAb 76-2-11 (αT8, IgG2α,κ), 74-12-4 (αT4, IgG2b,κ) and 76-6-7 (IgMκ), described by Pescovitz et al. (5, 6), were obtained from the American Type Culture Collection (Rockville, MD). Isotypes of mAb were determined by using monospecific antisera (Nordic Immunology, Tilburg, The Netherlands).

Purification and biotin conjugation of mAb. mAb prepared as ascites fluid were diluted 1/5 with 0.1 M phosphate buffer, pH 8, were filtrated through 0.2-μm filters, and were passed over protein A-Sepharose columns (Pharmacia, Uppsala, Sweden). mAb 295/33 (αT8) was eluted with 0.1 M Na-citrate, pH 4.5; the mAb 8/1, 305/44, and 74-12-4 (αT4) were eluted with 0.1 M Na-citrate, pH 3. The mAb fractions were neutralized with 1 M Tris and were dialyzed against 0.1 M $NaHCO_3$, pH 7.7, before concentration by ultrafiltration to 1 mg/ml protein (Millipore Immersible CX30™ ultrafiltration units; Millipore Corp., Bedford, MA).

For biotin conjugation, 1 ml of purified mAb (1 mg/ml in 0.1 M $NaHCO_3$, pH 7.7) was incubated for 30 min at 20°C with 0.05 ml of a freshly prepared solution of 1 mg (+) biotin-n-hydroxy-succinimide-ester (Calbiochem, Frankfurt, FRG) dissolved in 1 ml *N*-methylformamide. Excess biotin was removed by gel filtration through a Sephadex G-25M column (Pharmacia). The protein eluate was di-

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[5] Abbreviations used in this paper: C, complement; CML, cell-mediated lysis; FMF, flow microfluorometry; mAb, monoclonal antibody(ies); MLC, mixed lymphocyte culture; PE, phycoerythrin; PBL, peripheral blood lymphocytes; PPD, purified protein derivative of tuberculin; $T_{C/S}$, cytolytic/suppressor subset of T lymphocytes; $T_{H/I}$, T lymphocyte helper/inducer subset.

alyzed against PBS and was stored at −20°C.

Cell separation procedures. Peripheral blood lymphocytes (PBL) were obtained by Lymphoprep (Nyegaard, Oslo, Norway) separation of heparinized whole blood. Suspensions of thymus, spleen, and lymph node cells were separated accordingly. Bone marrow cells were obtained by rinsing porcine sterna with cold PBS.

Nylon wool-nonadherent cells were enriched by passage through syringes filled with nylon fiber (Fenwal Laboratories, Morton Grove, IL) (14). The nonadherent fraction contained less than 5% surface Ig-bearing cells, as determined by indirect immunofluorescence after staining with rabbit anti-pig Ig antisera (Miles Laboratories, Elkhart, IN) and FITC-conjugated goat anti-rabbit Ig (Cappel Laboratories, Cochranville, PA).

Immunofluorescence staining; dual-parameter flow microfluorometry (FMF). Lymphoid cells (2 to 3×10^6) were reacted with 0.1 ml of saturating concentrations of mAb diluted in PBS supplemented with 10 mM EDTA, 4% FCS, 20 mM HEPES, and 0.1% NaN_3 (FACS buffer). After 30 min on ice, cells were washed and were incubated for an additional 30 min in 0.1 ml FITC-conjugated goat anti-mouse IgG $F(ab')_2$ (diluted 1/60; Jackson Laboratories, Avondale, PA). Cells were washed three times with FACS buffer and were kept on ice until FMF analysis or microscopic inspection.

For dual-parameter FMF, FITC-labeled cells were further incubated with biotinylated mAb for 30 min at 4°C, and were then washed. Finally, 0.02 ml of phycoerythrin (PE)-conjugated avidin (Becton Dickinson, Mountain View, CA) was added for 20 min at 4°C. After washing, cells were analyzed on a FACS IV (Becton Dickinson, Sunnyvale, CA).

The fluorescence on cells was excited at 488 nm with a 4 W Argon laser. The green fluorescence of the FITC-labeled cells was measured through a 520 nm band pass filter and the yellow fluorescence of the PE-labeled cells through a 570 nm band pass filter (Oriel). The yellow (PE) and green (FITC) components of the emitted light were separated by a beamsplitter with a dichroic (560 nm) filter. A differential amplifier was used to correct for overlap of the green fluorescence emission spectrum of FITC into the yellow fluorescence emission spectrum of PE. The amplifier was adjusted so that the signals from cells stained only with FITC were orthogonal to the yellow fluorescence axis of the dual-parameter (yellow and green) fluorescence display. The exclusive analysis of lymphocytes was assured by setting electronic windows on the lymphocyte subpopulation in the dual-parameter display of right vs forward angle scatter.

Depletion of lymphocyte subpopulations with mAb plus complement (C). Cells (1 to 2×10^5 cells/well) cultured in round-bottomed microtiter plates (Greiner, Nürtingen, FRG) were sedimented for 10 min at 600 × G. The culture medium was collected, and the cells were resuspended in 50 μl of ascites (dilution 1/100 to 1/200) for 20 min at room temperature. After the addition of rabbit C (50 μl/well; final dilution 1/15 to 1/60) and incubation for 45 min at 37°C, cells were washed and were subjected to a second cycle of antibody and C treatment. Cells were finally resuspended in the original culture medium to continue cultivation.

Lymphocyte proliferation assay. PBL (2×10^6/ml) were suspended in RPMI 1640 (supplemented with 10% FCS, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES buffer, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin sulfate), and volumes of 100 μl were distributed into round-bottomed microtiter plates (Greiner). For activation with mitogen, concanavalin A (Con A; Pharmacia) was added (10 μg/ml). In vivo sensitization of pigs to tuberculin was achieved by subcutaneous injection of 10 ml complete Freund's adjuvant. PBL from primed animals and normal controls were restimulated in vitro with 500 IU of purified protein derivative of tuberculin (PPD) per ml (a gift from Behring-Werke, Marburg, FRG). Alloantigen-specific cells were activated in a mixed lymphocyte culture (MLC) by incubation of 2×10^5 PBL with the same number of γ-irradiated (30 Gy) allogeneic PBL.

For the determination of the proliferative response, 1 μCi of [^{3}H] thymidine (Amersham, Braunschweig, FRG) was added per well, and 18 hr later, the microcultures were harvested by using a Skatron multiharvester (Skatron, Lierbyen, Norway). [^{3}H]thymidine uptake was measured by liquid scintillation counting.

Cell-mediated lysis. The cytolytic activity of effector cells generated during an MLC was determined by using as target cells 3-day Con A lymphoblasts derived from the stimulator cell donor. Responder type Con A blasts served as controls. Target cells were labeled with $Na^{51}CrO_4$ and were incubated for 3 hr along with effector cells at various effector to target cell ratios. The percentage of specific cytolytic activity was calculated as described (4).

RESULTS

Reactivity of mAb 8/1 with lymphoid cells. For various lymphoid organs and for peripheral blood, the proportion of cells stained with mAb 8/1 was compared with the proportion of cells stained with antibodies that define the porcine $T_{C/S}$ ($CD8^+$) and $T_{H/I}$ ($CD4^+$) subsets of T lymphocytes, and with mAb 74-22-15 (5), which characterizes cells of the monocyte/macrophage lineage (Table I). The data indicate that mAb 8/1 detects an antigen, referred to as the 8/1 antigen, that is shared between thymocytes, both subpopulations of mature T lymphocytes, and monocytes, but absent on B lymphocytes. Erythrocytes and platelets do not express this antigen (not shown). The number of 8/1 antigen-positive cells in the bone marrow roughly equals the number of bone marrow cells stained with mAb 74-22-15, suggesting that these cells belong to the myeloid lineage. The same reactivity pattern was found with another mAb, mAb 305/44 (not shown).

Single-parameter FMF analysis (Fig. 1) of bone marrow cells revealed heterogeneity with regard to staining with mAb 8/1, whereas thymocytes were virtually all stained brightly. T lymphocytes in peripheral blood were stained with an intensity similar to that of thymocytes. Lymph nodes and spleen comprised cells with different 8/1 antigen density with a peak fraction showing a fluorescence intensity comparable to that of thymocytes and peripheral blood T lymphocytes.

These findings could suggest a constant 8/1 antigen density on T lymphocytes. Staining of mitogen (Con A)-induced T lymphoblasts with mAb 8/1, however, revealed a low antigen density, thus demonstrating that the expression of the 8/1 antigen is not constant, but can vary in T lymphocytes. Mitogen appeared to be essential for the decrease of 8/1 antigen density because mere cultivation of T lymphocytes in the absence of the stimulant did not result in reduced 8/1 antigen expression (Fig. 2).

Functional studies. The low 8/1 antigen density observed on Con A lymphoblasts raised the question of whether loss of the 8/1 antigen is a consequence only of polyclonal activation with mitogen or can be regarded as indicative of activation in general, including the generation of antigen-specific effector cells. To approach this question, T lymphocytes were subjected to different stimuli, and functional studies were performed to trace the expression of the 8/1 antigen during activation.

Treatment of PBL with mAb 8/1 and C before activation removed $8/1^+$ cells quantitatively (FMF analysis not shown) and abolished the ability to mount a proliferative response to mitogen (Con A), alloantigen, or soluble antigen such as PPD (Fig. 3). This result was caused by elimination of precursor cells and not only by depletion of $8/1^+$ antigen-presenting cells of the monocyte/macrophage lineage, because reconstitution with T4/T8-depleted PBL did not restore the proliferative response (Fig. 3, circles).

Different results were obtained when cells were treated at various times during activation (Fig. 3). The experiments were carried out without correction of cell numbers and without re-addition of the stimulant. Proliferative activity was determined at day 4 (Con A) or day 5 (MLC, PPD) of in vitro culture. It was found that already 1 day after sensitization, cytotoxic treatment no longer abolished the proliferative response completely, and thymidine incorporation of cells treated at day 3 or 4 reached the level observed in control populations treated with C

TABLE I

Distribution of lymphocyte surface markers

Antigens	mAb	% Positive Cells[a] in							
		Bone Marrow	Thymus	Spleen	Lymph Nodes	Peripheral Blood			
						Leukocytes	T Lympho-cytes	B(Ig⁺) Lym-phocytes	Monocytes
CD8 (T8)	αSL2.[b]αPT8[c]	<5	70–80	15–24	23–40	28–46	40–50	<5	<5
CD4 (T4)	αPT4[c]	<5	>60	n.d.[d]	40–50	34–50	40–50	<5	<5
—	74-22-15[c]	30–40	<5	n.d.[d]	n.d.[d]	10–18	<5	<5	>95
8/1	α8/1	20–50	>95	32–43	51–80	70–90	>95	<5	>95

[a] Data refer to five individuals.
[b] See Reference 4.
[c] See Reference 5.
[d] Not done.

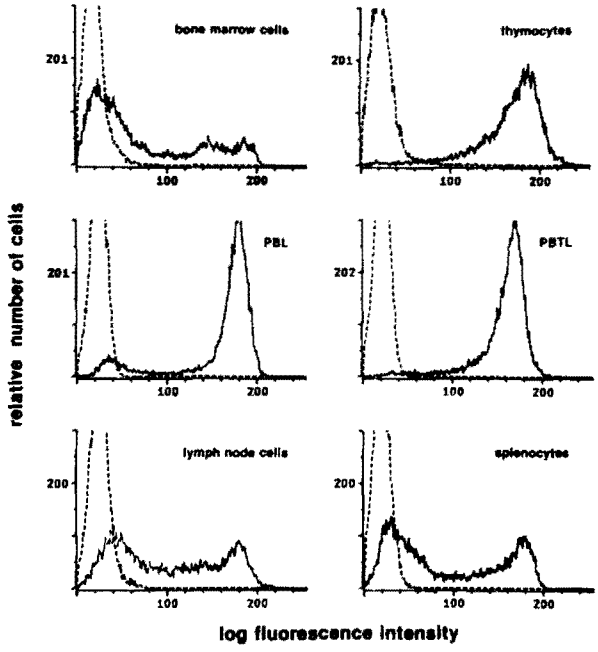

Figure 1. Distribution of the 8/1 antigen. Single-parameter FMF profiles of different lymphoid cell populations (PBTL, nylon wool-nonadherent peripheral blood T lymphocytes) stained with mAb 8/1 and FITC-conjugated goat anti-mouse IgG F(ab')₂. Labeling with second antibody alone served as negative control (dashed lines). Relative fluorescence intensities are expressed on a log scale.

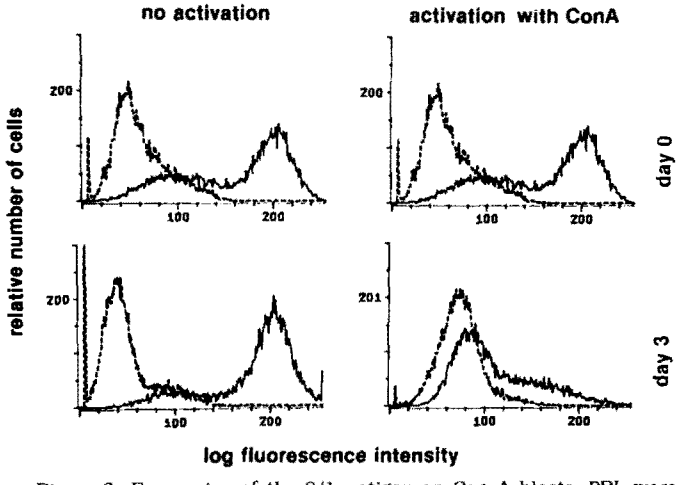

Figure 2. Expression of the 8/1 antigen on Con A blasts. PBL were either cultured in medium alone or stimulated with Con A (10 μg/ml). After 3 days, cells were stained with mAb 8/1 and FITC-conjugated goat anti-mouse IgG F(ab')₂, and 10^4 viable cells were analyzed by single-parameter FMF. Relative fluorescence intensities are expressed on a log scale.

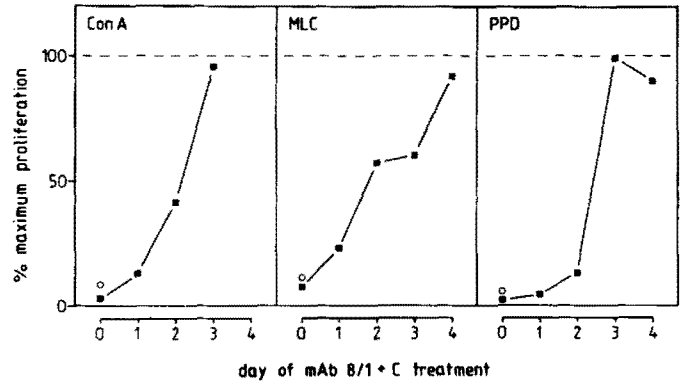

Figure 3. Expression of the 8/1 antigen in proliferating lymphocyte populations. PBL (2×10^6) were stimulated with either 10 μg of Con A/ml (Con A), with 2×10^5 γ-irradiated allogeneic PBL in an MLC (MLC), or with 500 IU of PPD/ml (PPD). Sets of replicate cultures were treated with mAb 8/1 and C either before sensitization or at the indicated times later. [³H]thymidine incorporation during an 18-hr pulse was measured from day 3 to day 4 after stimulation with Con A, and from day 4 to day 5 after sensitization with alloantigen or PPD. The data represent the mean cpm values of 12 replicate cultures normalized to the incorporation of cells treated at the respective times with C alone. These control values ranged from 77,600 to 85,900 cpm (Con A), from 8100 to 14,600 cpm (MLC), and from 6300 to 8200 cpm (PPD). Circles represent mAb 8/1 plus C-treated cultures that were reconstituted with antigen-presenting cells by adding the equivalent amount of T4/T8-depleted PBL.

alone. It can therefore be concluded that the responder cells gradually lost the 8/1 antigen and, after a few days of exposure to mitogen or antigen, finally acquired the $8/1^-$ phenotype. In this assay, the $8/1^-$ phenotype is defined by resistance to cytotoxic treatment.

It should be noted that the addition of mAb 8/1 to the cultures neither stimulated the lymphocytes nor inhibited the activation by mitogen or antigen. As mentioned above, proliferation was not observed in the MLC when the responder cells were treated with mAb 8/1 and C before activation with allogeneic stimulator cells, and, consequently, cytolytic effector cells were not generated in that case (not shown).

To test the phenotype of the CTL effector cells generated during a 7-day MLC, the effector population was treated with mAb and C before the cell-mediated lysis (CML) assay (Fig. 4). Whereas cytolytic activity was completely abolished after treatment with an αT8 mAb, substantial cytolytic activity was retained after treatment with mAb 8/1, thus demonstrating the existence of CTL effector cells of the $8/1^-T8^+$ surface phenotype.

Dual-parameter FMF analysis of 8/1 antigen expression. The functional data have indicated that CTL lose the 8/1 antigen in the course of activation. To confirm this loss of 8/1 surface expression and to include the $T_{H/I}$ subset in the analysis, the reactivity of mAb 8/1 with

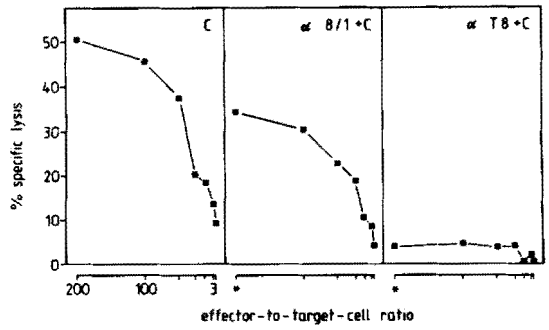

Figure 4. Serologic phenotype of CTL effectors. MLC-derived CTL were treated with mAb (mAb 8/1 and αT8 mAb 295/33, respectively) and C in the effector stage before the CML assay that was performed with target cells of the stimulator type. For $8/1^-$ and $T8^-$ effector cells, the effector to target cell (E:T) ratio was not adjusted to 200:1, and thus represents the authentic proportion of these cells (20% $8/1^-$ and 50% $T8^-$ cells, respectively) present in the control population treated with C alone (*number of cells equivalent to the E:T ratio of 200:1 of the C control). For each of the indicated E:T ratios (1000 ^{51}Cr-labeled target cells), the mean value of specific lysis in four replicates is given. Target cells of the responder type were not lysed.

$T8^+$ and $T4^+$ lymphocytes was examined by dual-parameter fluorescence analysis. $T_{C/S}$ and $T_{H/I}$ cells were labeled with αT8 and αT4 mAb, respectively. Fluorescent staining was achieved by the addition of FITC-conjugated second antibody. $8/1^+$ cells were detected by yellow fluorescence, using the biotinylated mAb 8/1 and PE-coupled avidin. Figure 5*A* shows the reactivity pattern of porcine PBL before and after activation with mitogen. In unstimulated $T_{C/S}$ lymphocytes stained with mAb 8/1 and αT8 (*top left*), five populations could be detected. Three of these exhibited a high intensity of staining with mAb 8/1, one of which did not express T8, while the other two were stained with low and high intensity, respectively. The existence of $T_{C/S}$ subpopulations with either low or high expression of T8 has been described (4). About 20% of the cells showed a low density of 8/1 and did not express T8. A small fraction of cells did not react with either antibody. After stimulation with Con A (*bottom left*), the high density T8 subset was $8/1^-$, whereas the subset with low T8 expression displayed a reduced but still detectable level of 8/1 antigen density.

In unstimulated $T_{H/I}$ lymphocytes stained with mAb 8/1 and αT4 (*top right*), three populations could be discriminated: one negative and one positive for T4, both of which were stained with mAb 8/1, and a third population that did not react with either antibody. The existence of populations negative for either T4 or T8 is concordant with the finding of Pescovitz et al. (6), namely that the expression of porcine T4 and T8 antigens is largely, although not completely, mutually exclusive. After activation with Con A, the $T4^+$ population was negative for 8/1 expression (*bottom right*). The fraction of $T4^-$ cells that express a low density of 8/1 (13%) probably represents those cells which show a low density of T8 and 8/1 (*bottom left*).

Similar results were obtained after testing the reactivity of mAb 8/1 with cells before and after MLC (Fig. 5*B*) or PPD stimulation (Fig. 5*C*). Note that cells analyzed at day 7 (bottom) were gated for lymphoblasts (approximately 20% of the populations). In studies in which the kinetics of 8/1 antigen expression was followed by single-parameter FMF, only a few cells in a population were found to respond to PPD or to alloantigen (data not shown). Therefore, to visualize the phenotypic change during specific activation of a minority of cells, the small resting cells that represent the majority had to be excluded from the analysis. In the MLC, cells of both functional subsets, $T_{C/S}$ and $T_{H/I}$, become activated. Accordingly, blastoid cells at day 7 of culture comprised $8/1^-T8^+$ and $8/1^-T4^+$ cells (Fig. 5*B*, *bottom*). Note that $8/1^-T8^+$ cells have been identified as CTL by the functional analysis shown in Figure 4. Stimulation by soluble antigen should predominantly activate the $T4^+$ $T_{H/I}$ subset. Accordingly, the number of $8/1^-T4^+$ cells exceeded the number of $8/1^-T8^+$ cells (Fig. 5*C*, *bottom*). In addition, the PBL population from the tuberculin-primed pig included a small but detectable subset (10%) of $8/1^-T4^+$ cells already before in vitro restimulation with PPD (Fig. 5*C*, *top right*). This could indicate that activation in vivo is also accompanied by down-regulation of the 8/1 antigen. Al-

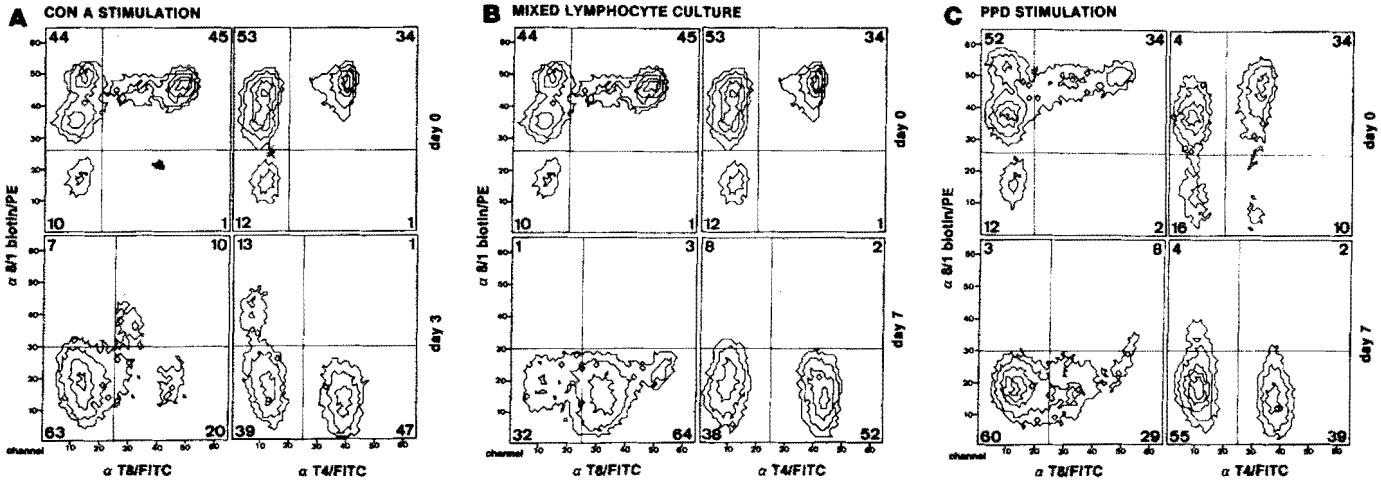

Figure 5. Dual-parameter FMF analysis of lymphocytes before and after in vitro activation. PBL derived from nonimmune (*A* and *B*) or tuberculin-primed (*C*) pigs were analyzed before in vitro activation and after stimulation with Con A for 3 days (*A*), with allogeneic stimulator cells for 7 days (*B*), or with PPD for 7 days (*C*). Cells were first stained indirectly with αT8 (295/33) or αT4 mAb and FITC-conjugated second antibody, followed by staining with biotinylated mAb 8/1 and PE-labeled avidin. On ordinate and abscissa, the relative log fluorescence intensities are expressed on a scale of 64 channels with 5×10^4 cells analyzed. Contour lines represent levels of 20, 40, 80, 120, and 160 cells. Unspecific staining of cells with PE-labeled avidin or FITC-conjugated second antibody was taken as a threshold for positive staining. For PBL (*top*, day 0), double-negative cells are included in the field below channel no. 26 (ordinate, PE-label) and to the left of channel no. 20 (abscissa, FITC-label). For lymphoblasts (*bottom*), the respective borderlines are represented by channels 30 and 25. The percentage of double-negative, double-positive, or single-positive cells is indicated by the number in one corner of the respective quadrant.

together, these data demonstrate that after activation, the 8/1 antigen expression is markedly reduced in both T cell subsets.

DISCUSSION

The murine mAb 8/1 recognizes an antigen present on virtually all porcine thymocytes and mature T lymphocytes, and, in addition, this antigen is also expressed on cells of the myeloid lineage. 8/1 antigen-positive T cells could be eliminated by antibody plus C treatment, and the antigen density determined by FACS analysis correlated with susceptibility to cytotoxic treatment (15, 16). Antibody alone had no effect on the proliferative response of T cells. The important finding was that porcine T4 and T8 lymphocytes both lose the 8/1 antigen after activation in vitro.

The 8/1 antigen could so far not be characterized biochemically. In the absence of biochemical data, the comparison with known mammalian leukocyte markers must be based on tissue distribution and functional properties. The ability to recognize an antigen that is present on monocytes and both T cell subsets, but is absent on B cells, erythrocytes, and platelets, is unique. Antibody OKM 1 has been reported to react with human macrophages, granulocytes, and T cells (17). Comparison of our FMF analysis with published data, however, reveals that 8/1 is expressed on all T lymphocytes at a density higher than that of T4 and T8, whereas the antigen detected by OKM 1 is expressed at a very low level only by a small fraction of $T8^+$ lymphocytes (17).

An mAb (76-6-7) which detects a surface marker with a distribution pattern similar to that of 8/1 on porcine leukocytes and which also failed to precipitate the antigen has been isolated by Pescovitz et al. (5). This suggested to us the possible identity of the antigens detected by mAb 8/1, 305/44, and 76-6-7. This suggestion was corroborated by the finding that both 305/44 and 76-6-7 competed efficiently with the binding of labeled mAb 8/1 to antigen (unpublished data).

The alteration of antigen expression during differentiation can also be used to compare lymphocyte surface antigens. Antigens down-regulated during development of murine T cells are detected by mAb B2A2 (9) and mAb J11d (18). For both of these antibodies, the respective antigen has not been characterized biochemically. Similar to 8/1, these antigens are expressed on thymocytes, macrophages, and polymorphonuclear cells but, different from mAb 8/1, these antibodies also bind to B cells and erythrocytes. It has been suggested that mAb B2A2 characterizes recent thymus emigrant cells which populate peripheral lymphoid organs (9). According to the distribution pattern of the B2A2 and the J11d antigens on T lymphocytes, these antigens appear to distinguish immature from mature T lymphocytes before activation, but not resting from activated T cells.

Expression of T lymphocyte surface antigens can be up- or down-regulated after activation by mitogen or specific stimulation by antigen. The majority of recirculating immunocompetent lymphocytes are arrested in the G_0 stage of the cell cycle (19), and activation by mitogen triggers lymphocytes to enter the cell cycle. Certain differentiation antigens undergo quantitative variations in the cell surface expression after activation. Down-regulation of 8/1 expression in the course of activation differs from the expression of the CD3, CD4, and CD8 antigens which increases during the G_1 phase (20). Likewise, the results exclude a relation to the family of molecules that are absent from resting cells and whose expression is up-regulated in a defined temporal sequence associated with the early, intermediate, late, and very late phase of T cell activation (10, 11).

Only a few antigens have been described whose expression declines after T cell activation. In its property to deplete all precursors of cytolytic T cells in the presence of C, but to spare cytolytic effector cells, mAb 8/1 is reminiscent of antibodies against the murine Lyt-1 antigen (12, 15), a member of the CD5 family (21) to which human T1 and rat MRC OX19 also belong. Different from CD5 antigens (1, 22) which are expressed at a low and heterogeneous density on thymocytes (1, 15, 22, 23), the 8/1 expression proved to be high and more homogeneous (Fig. 1). The expression of Lyt-1 is substantially enhanced on mature $T_{H/I}$ cells (15), whereas the level of 8/1 expression is comparable on thymic and post-thymic cells. Thus, not only the homogeneous expression of 8/1 on both subsets of porcine peripheral T lymphocytes but also the increase in Lyt-1 antigen expression during T cell maturation and the continuous expression on $T_{H/I}$ cells after activation distinguish Lyt-1 from 8/1.

Another surface molecule present on resting T lymphocytes but down-regulated on activated T lymphocytes is the lymphocyte homing receptor that permits adherence to the post-capillary venules of lymphoid organs which bear high-walled endothelium. On murine lymphocytes, this receptor is detected by mAb MEL-14 (13, 24). After antigen-specific or lectin-mediated stimulation, T cells temporarily lose homing receptors, including the MEL-14 determinant (13). Different from $8/1^+$ cells, however, the $MEL\text{-}14^{hi}$ T cells represent only a minor fraction of normal thymocytes, and the level of MEL-14 expression is 10 times higher on lymph node resident cells. Thus, although inhibition of porcine T lymphocyte homing by mAb 8/1 has not been studied, the level of antigen expression and the distribution of antigen-positive cells argue against the hypothesis that mAb 8/1 detects a porcine lymphocyte homing receptor.

The physiological role of the 8/1 antigen is unknown. It is not clear whether the expression is related to the cell cycle, because the acquisition of the $8/1^-$ phenotype requires about 3 days after mitogen stimulation. The question of whether the antigen is transiently or irreversibly down-regulated after activation is currently under study. Already at this stage of experimentation to the cellular immunologist, mAb 8/1 is a potentially useful tool. This antibody can be used to differentiate between B and T lymphocytes and, more important, the antibody discriminates resting from activated T lymphocytes in both subsets. It remains to be investigated whether this also applies to immune responses in vivo. So far, this is only indicated by the detection of an $8/1^-$ $T4^+$ population in tuberculin-primed pigs.

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