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Immunology

Monoclonal antibodies specific for murine CD2 reveal its presence on B as well as T cells

(mouse/transfectants/lymphocyte antigens/fluorescence-activated cell sorter)

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ABSTRACT **Monoclonal antibodies specific for the murine CD2 antigen were identified by an efficient screening method utilizing murine CD2 cDNA transfectants. An unexpected expression of CD2 on murine B cells was revealed by immunofluorescence and immunoprecipitation studies with these monoclonal antibodies and by RNA blot analysis for the murine CD2 transcript.**

The CD2 antigen, originally defined as the human T-lymphocyte antigen T11/erythrocyte-rosette receptor, has recently been the subject of extensive studies because of its involvement in T-cell recognition/activation. CD2 is thought to play a dual role in antigen-induced responses of T cells. First, CD2 is involved in the antigen-independent adhesion process as a ligand-binding molecule (1, 2) and, thus, facilitates the interaction between T cells and antigen-presenting cells (3, 4). Second, CD2 is involved in the alternative pathway of T-cell activation (5, 6) and, upon ligand-binding, may transduce an activating signal (7–11). The molecular properties of human CD2 characterized by cDNA cloning fulfill these biological functions (12, 13).

An important role of CD2 during intrathymic differentiation has been proposed on the basis of several lines of studies. A natural ligand for CD2 has been identified as lymphocyte function-associated antigen 3 (LFA-3; refs. 14–17), which is widely distributed (18–20) and also present on thymic epithelial cells (21). The interaction of CD2 on thymocytes with LFA-3 on thymic stromal cells is considered to be of major importance in the expansion of immature thymocytes, and this pathway of thymocyte activation is thought to come under regulation by the CD3-mediated signal in later stages of maturation (21–26).

In addition to these functions in T cells, CD2-mediated adhesion and signaling also appear to be involved in natural killer (NK) cell–target cell interaction (27–30). It remains controversial whether the CD2-mediated alternative pathway of T-cell activation can be driven independently of the CD3-mediated pathway (31–34). NK cells bearing no CD3–T-cell receptor complex are a suitable subject for investigating CD2-mediated signaling.

Studies concerning the biological functions of CD2 have been performed mainly in humans, except for some studies in rats. However, murine systems are superior for defining the physiological importance of CD2, as its putative roles during T-cell development could be examined directly during thymic ontogeny and in organ cultures of the fetal thymus. Moreover, mutant strains such as nude mice and *scid* mice are useful for investigating extrathymic development of T cells or NK cells, or both. In spite of such great advantages over human systems, CD2 studies in mice have been hampered by the lack of antibodies against murine CD2. Recently, we and others have cloned murine CD2 cDNA encoding a highly homologous protein to the human and rat CD2 molecule (35–37). Here we report the establishment of monoclonal antibodies (mAbs) against murine CD2. These mAbs were generated by immunizing rats and hamsters with murine thymocytes and were identified by an efficient screening system in which murine CD2 cDNA transfectants of the same species as the immunized animal were utilized. The strategy described here can be generally applied to identify antibodies reactive with cell-surface antigens encoded by cloned genes. In addition, expression of CD2 on murine B cells was revealed by these mAbs, which was unexpected from the studies on human CD2.

MATERIALS AND METHODS

Cell Lines. Hamster fibroblast tk^-ts13 cells obtained from American Type Culture Collection, rat fibroblast 3Y1 cells (38) kindly donated by K. Yokoyama (Riken, Tsukuba, Japan), murine fibroblasts NIH 3T3 and Ltk^-, and the transfectants derived from them were maintained in monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum. Cells were harvested by using trypsin/EDTA in phosphate-buffered saline. All other cell lines used were maintained in suspension cultures in RPMI 1640 medium supplemented with 10% fetal calf serum.

Establishment of Murine CD2 Transfectants. The full-length murine CD2 cDNA [1.2-kilobase (kb) *Eco*RI fragment prepared from pMCD2-2] (37) was introduced by blunt-end ligation at the unique *Xho* I site of a bovine papilloma virus-based expression vector, BMGneo (39). BMGneo, which has been successfully used for expressing cDNA encoding murine interleukins in mammalian cells, was used because of its property of stable extrachromosomal propagation, which often results in a high production of recombinant proteins. Transfection into fibroblasts was performed by the standard calcium phosphate coprecipitation method (40). Subconfluent monolayer cells were introduced with 5 μg of BMGneo-MCD2 plasmid DNA. Transfection into the murine myeloma P3X63-Ag8.653 was performed by the protoplast fusion method as described (39). Stable transformants were selected in medium supplemented with G418 (1 mg/ml) and then cloned by limiting dilution.

RNA Blot Analysis. Preparation of cytoplasmic RNA, denaturation, electrophoresis in 1% agarose gel containing formaldehyde, and transfer to nylon membrane were performed as described (37). cDNA encoding murine CD2

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Abbreviations: FACS, fluorescence-activated cell sorter; mAb, monoclonal antibody; LFA-3, lymphocyte function-associated antigen 3; NK, natural killer; Sp-B and Sp-T cells, splenic B and T cells; FITC, fluorescein isothiocyanate; PE, phycoerythrin; NP-40, Nonidet P-40.

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[850-base-pair (bp) *Eco*RI fragment prepared from pMCD2-2] (37) was subcloned into the pSP65 vector (Promega) containing the phage SP6 promotor (41). A ^{32}P-labeled single-stranded RNA probe complementary to mRNA was prepared from the linearized plasmid by using phage SP6 polymerase (Amersham) as described (41). Filters were hybridized overnight with the ^{32}P-labeled complementary single-stranded RNA probe in 50% formamide/6× SSPE/0.04% Ficoll/0.04% polyvinylpyrrolidone/0.04% bovine serum albumin/0.5% NaDodSO$_4$/100 μg of salmon sperm DNA per ml at 65°C (1× SSPE = 180 mM NaCl/10 mM sodium phosphate, pH 7.7/1 mM EDTA). The filters were washed four times with 0.1× SSPE/0.1% NaDodSO$_4$ for 15 min each at 65°C.

Preparation of Purified T and B Cells. Splenic T (Sp-T) cells were prepared from BALB/c spleen cells by two passages through a nylon wool column (42) and consisted of 90% Thy-1$^+$ cells and 6% B220$^+$ cells, as estimated by staining with mAb 30-H12 (anti-Thy-1) (43) and mAb RA3-6B2 (anti-B220) (44), respectively. Splenic B (Sp-B) cells were prepared from BALB/c spleen cells by complement-mediated lysis of T and NK cells. Briefly, spleen cells were first incubated with a mixture of anti-Thy-1 mAb (J1j) (45) and rabbit anti-asialoganglioside GM1 antibody (Wako Pure Chemicals, Osaka, Japan) and then incubated with rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada). The residual cells consisted of 3% CD3$^+$ cells and 95% B220$^+$ cells as estimated by staining with mAbs 145-2C11 (anti-CD3) (46) and RA3-6B2, respectively.

Immunofluorescence. Cells were incubated with polyclonal antiserum at a 1:100 dilution or with hybridoma culture supernatants. Preimmune serum at the same dilution was used as a negative control for the polyclonal antiserum. Bound antibodies were stained with fluorescein isothiocyanate (FITC)-labeled rabbit anti-hamster IgG (Cappel Laboratories) or FITC-labeled goat anti-rat IgG (UBI-Olympus, Tokyo). For two-color analysis, spleen cells were incubated with FITC- or phycoerythrin (PE)-labeled anti-murine CD2 mAb (RM2-2) in combination with PE-labeled anti-Thy-1 mAb (30-H12) or FITC-labeled anti-B220 mAb (RA3-6B2). The mAbs used were purified from ascites by DEAE-cellulose chromatography and then labeled with FITC or PE by standard procedures in our laboratory. Immunofluorescence was analyzed on a fluorescence-activated cell sorter (FACStar; Becton Dickinson).

Immunoprecipitation. Transfectants (2 × 10^7 cells) and thymocytes (2 × 10^8 cells) were surface-radioiodinated by the lactoperoxidase–glucose oxidase method (47). Iodinated cells first were incubated with polyclonal antisera at a 1:100 dilution or with hybridoma culture supernatants. The cells were washed with phosphate-buffered saline and then extracted with 1% Nonidet P-40 (NP-40)/50 mM Tris·HCl, pH 7.4/150 mM NaCl/1 mM phenylmethylsulfonyl fluoride/0.05% sodium azide (lysis buffer). Immunocomplexes were precipitated with protein A-Sepharose (Pharmacia) precoated with rabbit anti-hamster IgG (Cappel Laboratories) or rabbit anti-rat IgG (Dako Japan, Kyoto). For immunoprecipitation from Sp-T and Sp-B cells, surface-radioiodinated cells (1 × 10^8 cells) were extracted with the lysis buffer, and radiolabeled CD2 was immunoprecipitated by RM2-2-conjugated Sepharose. Control precipitates were obtained with 30-H12 (anti-Thy-1)-conjugated Sepharose. Immunoprecipitates were washed with 0.5% NP-40/0.5% sodium deoxycholate/50 mM Tris·HCl, pH 7.4/150 mM NaCl/0.05% sodium azide and then subjected to NaDodSO$_4$/10% PAGE followed by autoradiography.

Immunization and Production of mAbs. Adult Syrian hamsters and SD rats (Sankyo Labo Service, Tokyo) were first primed by an intradermal injection of BALB/c thymocytes (1 × 10^7 cells) emulsified in complete Freund's adjuvant, given a booster injection 10 days later with the same number of thymocytes in incomplete Freund's adjuvant, and injected i.p. three times weekly with thymocytes (1 × 10^7 cells) in phosphate-buffered saline. After 10 days, polyclonal antisera were collected, and antibody activities against murine CD2 transfectants were estimated by immunofluorescence. Animals developing a high titer of antibody were finally given an i.p. injection of murine thymocytes (2 × 10^7 cells). Immune splenocytes were prepared 4 days later and fused with P3U1 murine myeloma cells (48). The initial screening of hybridoma supernatants was performed by means of cell ELISA. Monolayers of murine CD2 cDNA-transfected fibroblasts briefly fixed with 0.025% glutaraldehyde in phosphate-buffered saline in 96-well plates were used as the target. Reactivities to the nontransfected fibroblasts were also estimated in parallel. Bound antibodies were detected by serial incubations with rabbit anti-hamster IgG (Cappel Laboratories) or rabbit anti-rat IgG (Dako), horseradish peroxidase-labeled protein A (Olympus), and the substrate *o*-phenylenediamine/H_2O_2.

RESULTS

Expression of Murine CD2 in Rat and Hamster Fibroblast. To efficiently discriminate the antibodies reactive with murine CD2 antigen from those reactive with other murine antigens, we took advantage of an artificial target antigen, the murine CD2 molecule expressed on fibroblasts of the same species as the antisera source. The murine CD2 cDNA was introduced into an expression vector BMGneo, and the resulting plasmid (BMGneo-MCD2) was used for the transformation of the rat fibroblast line 3Y1 and the hamster fibroblast line tk$^-$ts13. After selection in medium supplemented with G418 for 2–3 weeks, cytoplasmic RNA prepared from the transformants was examined for the transcript originating from the introduced murine CD2 cDNA on RNA blot analysis. A transcript of 1.8 kb, 0.5 kb longer than the natural murine CD2 transcript of 1.3 kb (37), was abundantly detected in the transformants but not in the recipients (data not shown). The increase in length may result from the addition of 5′ and 3′ untranslated sequences derived from the rabbit β-globin gene in the expression vector BMGneo (39). The transformants were then cloned by limiting dilution in medium supplemented with G418, and the resulting clones were screened for the murine CD2 transcript by dot-blot analysis. Several clones with various amounts of the transcript were established and subjected to subsequent immunofluorescence and biochemical analyses.

Hamster or Rat Antisera Raised Against Murine Thymocytes Show Reactivity to the Murine CD2 Antigen. Several hamsters and rats were immunized with murine thymocytes to develop antibodies against the murine CD2 antigen. Murine thymocytes were preferred as the immunogen because our previous study had indicated an abundant murine CD2 transcript in thymocytes (37). The hamsters and rats were bled 1 week after the fourth booster immunization, and the sera were examined by immunofluorescence for reactivity to the murine CD2 transfectants. Fig. 1 shows a representative result of the staining with the hamster antiserum of two cloned hamster transfectants, whose transcriptional levels of murine CD2 differed by about 10-fold. The antiserum preferentially reacted with the transfectants, and the cell-surface fluorescence intensity detected for each clone correlated with the transcriptional level of the murine CD2. Similar results were also obtained for the rat antisera. Preferential reactivities to the murine CD2 transfectant originating from 3Y1 were evident (data not shown).

We next identified the surface molecule(s) on the murine CD2 transfectants reactive with these antisera by immunoprecipitation using NP-40 extracts of the surface-radioiodinated transfectants followed by analysis on NaDodSO$_4$/

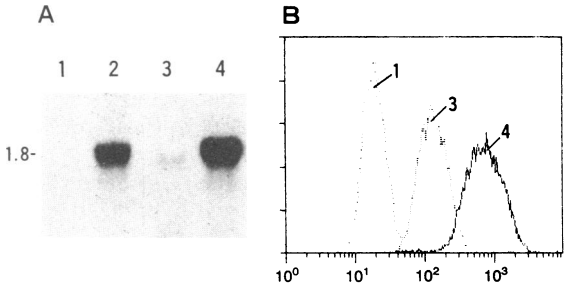

FIG. 1. Hamster anti-murine thymocyte antiserum showed reactivity to the murine CD2 transfectants. (*A*) RNA blot analysis for the transcriptional levels of the introduced murine CD2 cDNA in two representative clones derived from the hamster tk⁻ts13–murine CD2 transfectants. Ten micrograms of each cytoplasmic RNA was loaded. RNA blot analysis was performed as described. Lanes: 1, tk⁻ts13 recipient; 2, bulk of hamster tk⁻ts13–murine CD2 transfectants; 3, clone A1; 4, clone A3. Size of the transcript is indicated in kb at the left. (*B*) Immunofluorescent analysis with hamster anti-murine thymocyte antiserum. Immunofluorescence and flow cytometric analysis were performed as described. The number on each histogram corresponds to lanes in the RNA blot analysis indicated in *A*.

PAGE (Fig. 2). Only a single band of 60–67 kDa was detected in the immunoprecipitates obtained from the hamster tk⁻ts13-murine CD2 transfectant with hamster antiserum. Similarly, a single band of 55–75 kDa was detected when rat antiserum was used against the rat 3Y1–murine CD2 transfectant. Recently, Sewell *et al.* reported that a candidate for the murine CD2 antigen, a glycoprotein of 55–66 kDa, was immunoprecipitated from the extract of surface-radioiodinated murine thymocytes with a polyclonal rabbit antiserum raised against the purified human CD2 antigen (35). The molecular size of the deglycosylated polypeptide (35 kDa) was consistent with the predicted size based on the murine CD2 cDNA sequence (35). We also observed that the bands indicated in Fig. 2 decreased to a band of 38 kDa after N-Glycanase treatment (data not shown). These findings strongly suggested that antibodies reactive with the murine CD2 antigen could be successfully raised in hamsters and rats by immunization with murine thymocytes.

Establishment of mAbs Specific for the Murine CD2 Antigen. Splenocytes prepared from immunized rats and hamsters whose antisera exhibited highly preferential reactivities to the murine CD2 transfectants were fused with murine myeloma cells P3U1 by a standard polyethylene glycol method. Culture supernatants of the resulting hybridomas were first screened by cell ELISA with glutaraldehyde-fixed target cell sheets of murine CD2 transfectants prepared in 96-well plates. Background reactivities to the recipient nontransfected fibroblasts were also examined in parallel. The hybridoma supernatants that exhibited preferential reactivity in ELISA against the murine CD2 transfectants were next subjected to a secondary screening by immunofluorescence on FACS. The hybridomas exhibiting positive staining were then cloned by limiting dilution. In this way, one hamster mAb (HM2-1) and two rat mAbs (RM2-1 and RM2-2) were established from two separate fusions. However, the hamster–mouse hybridoma HM2-1 was unstable and ceased to produce antibody after a month. All of these mAbs specifically reacted with the murine CD2 transfectants established from tk⁻ts13, 3Y1, NIH 3T3, Ltk⁻, and P3X63-Ag8.653 cells but not with the recipient nontransfected cells (data not shown). Two murine T lymphomas, EL-4 and RLδ1, containing abundant CD2 transcript (37) were strongly reactive; the thymoma BW5147 containing a lesser amount of the transcript (37) was marginally reactive; and the mastocytoma P815 containing no CD2 transcript (37) was not reactive with these mAbs on FACS analysis (data not shown).

These three mAbs also strongly reacted with up to 95% of murine thymocytes (data not shown). Fig. 3 shows the $NaDodSO_4$/PAGE analysis of the immunoprecipitates obtained from the extracts of surface-radioiodinated rat 3Y1–murine CD2 transfectant (Fig. 3*A*) or of murine thymocytes (Fig. 3*B*) with mAbs RM2-1 and RM2-2. The polyclonal rat anti-murine thymocyte antiserum used in Fig. 2 was also included as a positive control. The single polypeptide of 55–70 kDa reactive with the polyclonal antiserum was also immunoprecipitated from the transfectant by mAbs RM2-1 and RM2-2. A broad band of 55–75 kDa was precipitated from murine thymocytes with mAbs RM2-1 and RM2-2. All of these bands obtained from the transfectants and thymocytes comigrated to form a sharp band of 38 kDa after N-Glycanase treatment (data not shown). These results clearly indicate that the mAbs RM2-1 and RM2-2 identify the murine CD2 antigen expressed on the surface of murine thymocytes.

Surface Expression of Murine CD2 on Sp-T and Sp-B Cells. By utilizing FITC- or PE-labeled RM2-2, we examined the

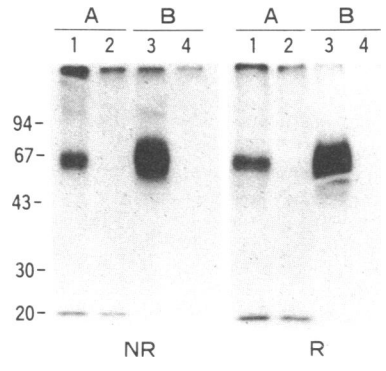

FIG. 2. Immunoprecipitation of the molecule reactive with anti-murine thymocyte antisera from the murine CD2 transfectants. Surface radioiodinated hamster tk⁻ts13–murine CD2, clone A3 (*A*) and rat 3Y1–murine CD2, clone B1 (*B*), were first incubated with the hamster antiserum (lane 1), preimmune hamster serum (lane 2), the rat antiserum (lane 3), or preimmune rat serum (lane 4). The cells were then washed and extracted with NP-40. Immunocomplexes were precipitated with protein A-Sepharose precoated with rabbit anti-hamster IgG (lanes 1 and 2) or rabbit anti-rat IgG (lanes 3 and 4), and subjected to $NaDodSO_4$/PAGE analysis under nonreducing (NR) or reducing (R) conditions. Molecular sizes of standards are indicated in kDa at the left.

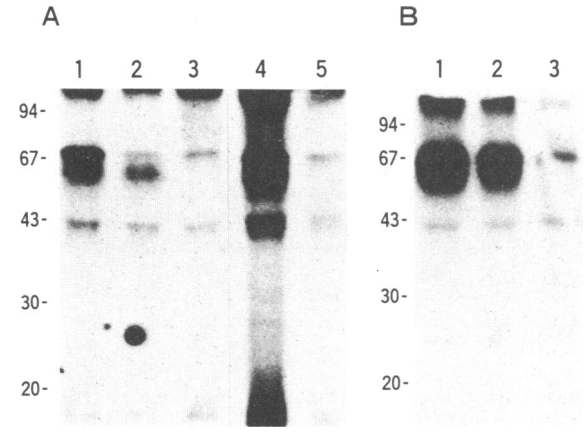

FIG. 3. Immunoprecipitation of the molecule defined with mAbs RM2-1 and RM2-2. Surface radioiodinated rat 3Y1–murine CD2, clone B1 (*A*), and murine thymocytes (*B*) were first incubated with the culture supernatant of RM2-1 (lane 1), culture supernatant of RM2-2 (lane 2), culture supernatant of P3U1 (lane 3), rat anti-murine thymocyte antiserum (lane 4), or preimmune rat serum (lane 5). Subsequent analysis was performed as described in Fig. 2.

surface expression of CD2 on murine spleen cells. Thy-1$^+$ T cells were brightly stained with RM2-2 (Fig. 4*A*) and almost all B220$^+$ B cells were also stained to a comparable extent (Fig. 4*B*). This unexpected reactivity with the majority of Sp-B cells was not due to a nonspecific binding of the labeled RM2-2 mAb in that the staining of both Thy-1$^+$ and B220$^+$ cells was only abolished by preincubation with an excess amount of unlabeled RM2-2 itself but not with normal rat serum or other rat mAbs including isotype-matched ones (rat IgG2a), and that the reactivity to both Thy-1$^+$ and B220$^+$ cells was completely depleted after absorption with the hamster tk$^-$ts13–murine CD2 transfectant but not with the recipient (data not shown). Although these results suggested that CD2 was also expressed on B cells in mice, it could still be argued that this reactivity may be due to an irrelevant crossreaction with some other molecule than CD2. To clarify this, we immunoprecipitated the reactive molecule from the purified Sp-B cells and compared it with that from the purified Sp-T cells. Similar bands of 55–70 kDa were detected for both Sp-T and Sp-B cells (Fig. 4*C*), and they comigrated to 38 kDa after N-Glycanase treatment (unpublished data). We finally confirmed the expression of CD2 in murine T and B cells by RNA blot analysis for the murine CD2 transcript. The murine CD2 transcript of 1.3 kb was readily detectable in Sp-B as well as in Sp-T cells (Fig. 4*D*). No Thy-1 and T-cell antigen receptor β chain transcripts were detected for this preparation of B cells after hybridization with the corresponding cDNA probes (data not shown), indicating that the CD2 transcript detected was not due to any contaminating T cells. These results clearly demonstrated that murine B cells as well as T cells express CD2.

DISCUSSION

Increasing evidence has revealed that many functional molecules of biological importance possess evolutionarily conserved primary structures. The recent progress in molecular biological techniques enables the isolation and characterization of homologous gene products in other species by cross-hybridization. Cells involved in immune reactions display a variety of surface molecules that are responsible for specific effector or recognition functions (49, 50). Various surface markers displayed during thymic ontogeny and those displayed on different subsets of mature T cells have been extensively characterized, mainly in humans and mice. Studies in these two species have often been carried out in a parallel and complementary manner so as to establish the physiological importance of the subject when an experimental system was difficult to establish for either one of the species.

The molecules of major importance in the T-cell recognition of antigen, such as the T-cell antigen receptor α, β, γ, and δ chains; the CD3 γ, δ, ε, and ζ chains; CD4; and CD8 have been cloned independently or by cross-hybridization in both species, and mAbs for most of them have been established (51–54). In addition, several accessory molecules are involved in the T-cell recognition/activation process (55, 56). Among them, LFA-1 and CD2 have been implicated in an antigen-independent conjugate formation process preceding the T-cell receptor-mediated antigen recognition (1–4, 57). mAbs against LFA-1 have been established and exhibit similar functional properties in both species (58, 59). In contrast, murine studies of CD2 have been hampered by the lack of mAb reactive with surface murine CD2.

To establish the mAb to murine CD2, we first cloned the murine CD2 cDNA by cross-hybridization with rat CD2 cDNA (37). Various mAbs reacting with lymphoid cells have so far been identified by first screening for specificity to a particular cell population of interest and then characterizing the biochemical and functional characteristics of the recognized antigen. However, such a classical strategy is not efficient for establishing mAbs to a particular molecule of interest. Therefore, we took advantage of the cDNA-mediated gene expression technique to make artificial target cells, a procedure that gives us an efficient way of discriminating antibodies to the murine CD2 antigen, as described herein. Recipient cells of the same species as the immunized animal were utilized so as to reduce interference of other antibodies such as those to murine H-2 antigen. Fibroblasts were preferred because of their high transformation efficiency and their easy applicability to cell ELISA. The high efficiency of this strategy was clearly demonstrated. We established three mAbs to the murine CD2 antigen from two fusions. Moreover, by analyzing the reactivities of the anti-murine CD2 mAbs, murine CD2 was unexpectedly expressed on the majority of B cells (Fig. 4). Thus, it became clear that it was impossible to identify a mAb to murine CD2 by the classical strategy based on the preferential reactivity to T cells. As has been well-demonstrated in the case of Thy-1 (60–62), lineage specificity of expression is not always conserved

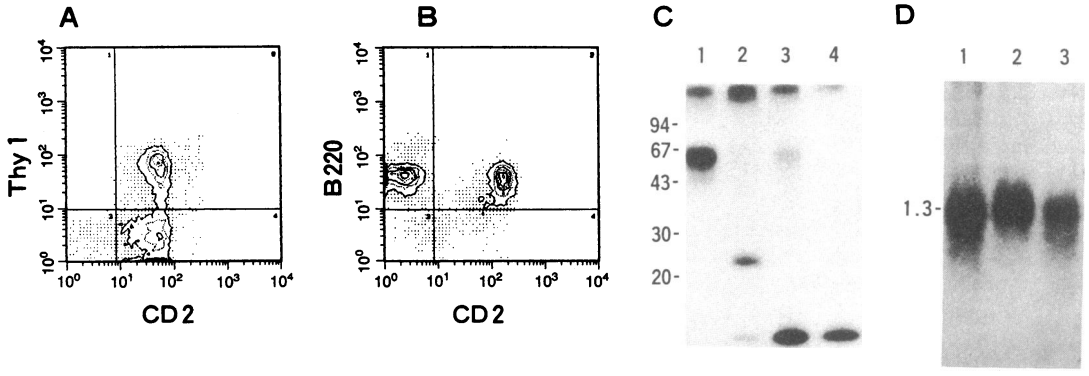

FIG. 4. Expression of CD2 in murine Sp-T and Sp-B cells. (*A* and *B*) Two-color immunofluorescent analysis. BALB/c spleen cells were incubated with FITC-labeled RM2-2 mAb (anti-CD2) plus PE-labeled 30-H12 mAb (anti-Thy-1) (*A*) or with PE-labeled RM2-2 mAb plus FITC-labeled RA3-6B2 mAb (anti-B220) (*B*), and then two-color immunofluorescence was analyzed on a FACStar. Ten thousand viable cells were analyzed in each sample. Markers were set against the contour plot of control samples, which involved substitution of diluent alone for either one or both antibodies. (*C*) Immunoprecipitation of the molecules defined with mAb RM2-2 from purified T and B cells. The NP-40 extracts of surface-radioiodinated Sp-T cells (lanes 1 and 2) and Sp-B cells (lanes 3 and 4) were incubated with RM2-2-conjugated Sepharose (lanes 1 and 3) or 30-H12-conjugated Sepharose (lanes 2 and 4). Immunoprecipitates were washed and then subjected to $NaDodSO_4$/PAGE analysis under reducing conditions. Molecular sizes of standards are indicated in kDa at the left. (*D*) RNA blot analysis for the murine CD2 transcript in purified T and B cells. Lanes: 1, total spleen cells; 2, purified Sp-T cells; 3, purified Sp-B cells. Ten micrograms of each cytoplasmic RNA was loaded. Size of the transcript is indicated in kb at the left.

between species. Our system also can serve as definitive proof of this.

In a similar way, we already have identified several new mAbs to the murine CD4 antigen (L3T4) in parallel with several additional mAbs to murine CD2 from the same fusions (unpublished data). The system described here should be very useful, especially when several new mAbs recognizing different epitopes are required to finely characterize the functional domains of a certain molecule.

The most unexpected finding from the use of the anti-murine CD2 mAbs was their substantial reactivity to B cells, as indicated in Fig. 4. In humans, CD2 has been recognized as a specific marker of cells of T and NK lineage (12, 13, 63–65); however, all three anti-murine CD2 mAbs described here exhibited a comparable reactivity to murine B and T cells. This expression of CD2 on murine B cells has also been demonstrated for bone marrow cells and some cell lines of B lineage by means of flow cytometric analysis, immunoprecipitation, and RNA blotting analysis (unpublished data).

In humans, several functional aspects of CD2 have been demonstrated in T and NK cell activation (5–11, 27–30). Disappointingly, so far no functional properties of RM2-1 and RM2-2 have been demonstrated in proliferative T-cell responses to phytohemagglutinin, Con A, or alloantigens and in NK cell-mediated cytotoxicity (unpublished data).

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