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T Cell-independent Antibody-mediated Clearance of Polyoma Virus in T Cell-deficient Mice

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Summary

Polyomavirus (PyV) infection of SCID mice, which lack functional T and B cells, leads to a lethal acute myeloproliferative disease (AMD) and to high levels of virus replication in several organs by two wk after infection. This is in contrast to infection of T cell–deficient athymic nude mice, which are resistant to acute PyV-induced disease and poorly replicate the virus in their organs. This major difference in the virus load and in the outcome of PyV infection between SCID and nude mice suggested that an efficient, T cell–independent antiviral mechanism operates in T cell–deficient, PyV-infected mice. To investigate this possibility, mice with different genetically engineered T and/or B cell deficiencies and SCID mice adoptively reconstituted with B and/or T cells were infected with PyV. The results indicated that the presence of B cells in the absence of T cells protected mice from the AMD, and this was accompanied by a major reduction of PyV in all organs tested. Sera from PyV-infected T cell receptor (TCR) $\alpha\beta$ knockout or TCR $\alpha\beta$ $\gamma\delta$ knockout mice contained IgG2a antibodies to PyV. Sera or purified immunoglobulin fractions from PyV-infected TCR $\alpha\beta$ knockout mice protected SCID mice from the PyV-induced AMD. To our knowledge, this is the first report of an effective T cell–independent antibody response clearing a virus and changing the outcome of infection from 100% mortality to 100% survival.

Virus-specific T cells usually play a major role in the process of recovery from acute virus infections (1). After an early phase of the infection, when nonspecific factors such as NK cells contribute to decreasing the virus load (2), MHC-restricted cytotoxic T cells locate and destroy virus-infected target cells. T cells also secrete cytokines, which further impair virus replication and participate in several aspects of the immune response (3). For the generation of a high-affinity antibody response and for efficient isotype switching from IgM to IgG and IgA production, T cell help is believed to be essential (4–6). In some virus infections, such as vesicular stomatitis virus (VSV)[1], reovirus, and Sindbis virus, humoral immunity in the presence of T cells can play a significant role in the control of infection (7–9). Of interest is that athymic nude mice can clear intracerebral Sindbis virus infection similarly to immunocompetent mice, suggesting that a T cell–independent immune response may restrict the replication of this pathogen (10, 11). Nude mice, however, are not completely free of T cells, and a role for T cells or T cell–dependent antibodies could not be excluded in those experiments. Efficient

control of an acute virus infection by antibodies produced in the absence of T cells has therefore not been shown.

Recent developments in immunology, using mice with targeted disruption (knockout) of certain genes, show that there are many redundancies in the immune system. Mice defective in gene products shown to be important for control of infections often can clear viruses or parasites with unexpected efficiency. For example, in mice defective in $CD8^+$ T cells, virus infections known to be controlled by $CD8^+$ CTL were cleared by compensating $CD4^+$ T cells (12, 13). A potent anti-lymphocytic choriomeningitis virus (LCMV) T cell response and VSV clearance in IFN-γ receptor knockout mice (14), detectable CTL responses to LCMV infection in IL-2 knockout mice (15), and the control of leishmaniasis in CD4 knockout mice (16) are further examples. This report shows that although T cells in the absence of B cells can provide full protection against polyomavirus (PyV) infection in mice, B cells can also clear PyV in a completely T cell–independent fashion, supporting the view that the immune system has components with overlapping functions, and providing the first unambiguous example of a protective T cell–independent antiviral antibody response.

PyV induces a lethal acute myeloproliferative disease (AMD) in SCID mice that lack functional T and B cells (17). This observation is surprising since hematological disorders are not part of the disease spectrum caused by PyV

infection in normal newborn or in T cell–deficient nude mice (18, 19). Moreover, the AMD in SCID mice is associated with high levels of PyV replication on day 14 after infection in all major organs tested, whereas in nude mice virus replication cannot be detected in most organs at this time (17, 20, 21). This enhanced virus replication and virus-induced pathology in SCID versus nude mice suggest that an alternative mechanism of PyV clearance is operating in mice deficient in T cells. Here we demonstrate that PyV infection induces virus-specific IgM and IgG production by B cells in the absence of T cell (and NK cell) help, and that this antibody response can clear PyV and protect T cell–deficient mice from the acute lethal myeloproliferative disease caused by the virus.

Materials and Methods

Mice and Virus Infection. C57BL/6JSz SCID mice, 129 TCR αβγδ−/− mice, which had a targeted disruption in their TCR β and δ genes (22) and therefore lacked functional αβ and γδ T cells, 129×C57BL/6 μ−/− mice, which had no functional B cells (23), 129×C57BL/6 and BALB/c mice were obtained from The Jackson Laboratory (Bar Harbor, ME). 129×C57BL/6 TCR αβ−/− mice, which had a targeted disruption of the TCR α or β gene (24), and therefore had no functional αβ T cells, had been backcrossed three-to-four times to C57BL/6. The TCR αβ−/− (initially from Drs. P. Mombaerts and S. Tonegawa, Massachusetts Institute of Technology, Cambridge, MA) and C.B17 SCID mice were bred in the Department of Animal Medicine of the University of Massachusetts Medical Center, and were housed in microisolator cages in this facility. Mice to be infected were inoculated intraperitoneally with 2 × 10^5–2 × 10^6 50% tissue culture infectious dose (TCID₅₀) of PyV strain A2, provided by Dr. Michele M. Fluck (Michigan State University, East Lansing, MI).

In Vivo NK or T Cell Depletion. Mice with the C57BL/6 background, which express the NK1.1 alloantigen, were depleted of NK cells by i.p. injection with 20–100 μl of previously determined optimal dilutions of ammonium sulfate cuts of ascites preparations of a monoclonal antibody to NK1.1 (25). C.B17 SCID mice and TCR αβγδ−/− mice, which do not express the NK1.1 alloantigen, were depleted of NK cells by an i.p. injection of 10 μl of antiserum to asialo GM1 (Wako Chemicals USA, Dallas, TX). The anti–NK1.1 or asialo GM1 injections were repeated weekly. The effectiveness of NK cell depletion was tested by using splenocytes of the depleted mice in cytotoxicity assays with ⁵¹chromium-labeled conventional NK target cells (YAC-1) in vitro.

CD4+ and CD8+ T cells were depleted in vivo by i.p. injection of 80 μl of an ascites preparation of a rat anti–mouse CD4 monoclonal antibody (clone GK1.5 [26]), and of 150 μl of a 1:10 dilution of an ascites preparation of a rat anti–mouse CD8 monoclonal antibody (clone 2.43 [27]), respectively. The injections were repeated every 6–7 d. The effectiveness of depletion was tested by FACS® (Becton Dickinson Co., Mountain View, CA) staining of peripheral blood leukocytes of killed mice, and these samples were found to be 98–99% free of CD4+ and CD8+ T cells.

Adoptive Transfer Experiments. Spleens harvested from mice were ground between the frosted ends of glass microscope slides; the resulting cell suspension was filtered through a nylon mesh and treated with 0.83% NH₄Cl to remove erythrocytes. Cells prepared from two-to-three spleens (with or without in vitro T cell depletion) were suspended in 650 μl PBS; 200 μl of this spleen cell suspension per mouse was given intravenously into C.B17 SCID mice. The adoptive transfer of cells was performed 1 d before the infection of the mice intraperitoneally with 2 × 10^5 TCID₅₀ units of PyV strain A2.

In Vitro T Cell Depletion. Depletion of CD4+ and CD8+ T cells in spleen cell preparations in vitro was performed by treating the cells with rat anti–mouse Thy-1 monoclonal antibody (clone J1J.10 rat ascites preparation [28]; 1:50 dilution, 150 μl/10^7 cells) on ice for 45 min, washing them twice with PBS, adding 25 μl/10^7 cells of guinea pig serum as a complement source, and then incubating the cells for 10 min on ice and for 50 min at 37°C. Finally, the cells were washed twice and suspended in medium.

Southern Blots. DNA was prepared from frozen organs (liver, lung, kidney, and spleen) following published protocols (29). 2–10 μg of undigested DNA or DNA cut with EcoRI, which cuts the PyV genome once, was subjected to electrophoresis in 0.8% agarose gels, and transferred to nitrocellulose paper by vacuum blotting (30), or to nylon transfer membrane (Magna Charge; Micron Separations Inc., Westboro, MA) by capillary transfer. The DNA blot was then baked, prehybridized, hybridized, washed, and exposed to autoradiography as described (31). PyV strain A2 cloned in pAT153 plasmid was kindly provided by Dr. Michele M. Fluck, and it was ³²P-labeled by random priming for use as a hybridization probe.

Immunofluorescent Staining. NIH 3T3 clone A31 cells were seeded on microscope glass coverslips and infected with PyV strain A2. On day 3 after infection, the cells on the coverslips were fixed for 10 min with a 1:1 mixture of ethanol and ethyl-ether, followed by 20 min in 95% ethanol. Staining was performed in a humid chamber for 35 min at room temperature with dilutions of sera from PvY-infected immunocompetent or αβ knockout mice or of normal (uninfected) mouse serum. The coverslips were subsequently rinsed with PBS and incubated for 35 min with FITC-labeled antibodies specific for mouse IgM (μ chain), IgG (γ chain) (Vector Laboratories, Inc., Burlingame, CA), IgG1, IgG2a, IgG2b, and IgG3 (Zymed Laboratories, Inc., San Francisco, CA). The coverslips were rinsed with PBS, mounted on microscope slides with 90% glycerol/10% PBS, and viewed by fluorescence microscopy.

Purification of IgG from PyV-infected αβ Knockout Mouse Serum. A protein A–Sepharose CL-4B (Pharmacia Biotech, Piscataway, NJ) column was prepared according to the manufacturer's instructions and washed with 0.1 M Tris-HCl, pH 8.0 (binding buffer). A 0.5-ml sample of pooled serum harvested on day 14 after PyV infection of αβ knockout mice was mixed with 50 μl 1 M Tris-HCl, pH 8.0, loaded on the column, and washed with binding buffer (10× column volume). The IgG fraction was eluted by 0.1 M citric acid, pH 3.0. To preserve the activity of acid-labile IgG, each collected fraction (0.5 ml) was neutralized by the addition of 50 μl 1 M Tris-HCl, pH 9.0. The protein content of the fractions was determined by an OD 280 reading, and the PyV-specific IgG content by indirect fluorescent staining.

Results

Outcome of PyV Infection in Mice Deficient in T and/or B Cells. We have shown that SCID mice developed a fatal AMD after infection with PyV. The incidence of the AMD (100%) and the survival time of the SCID mice (14–16 d) was not influenced by the genetic background of the mice or

Table 1. *PyV-induced Disease in Immunodeficient Mice*

Mice	No. of mice	Depletion (in vivo)	Killed* (day)	Disease
C.B17 SCID	15	−	13.6 ± 0.5[‡]	AMD
C57BL/6 SCID	22	−	13.9 ± 1.3	AMD
C.B17 SCID	5	αAGM1	14.2 ± 0.8	AMD
C57BL/6 SCID	22	αNK1.1	13.9 ± 1.3	AMD
TCR αβ −/−	3	−	28(1)[§]	GI
			69(1)	GI
			87(1)	Healthy
TCR αβ−/−	4	αNK1.1	67(1)	GI
			87(3)	Healthy
TCR αβγδ−/−	4	−	14(2)	Healthy
			42(2)	Healthy
TCR αβγδ−/−	5	αAGM1	14(2)	Healthy
			42(3)	Healthy
μ−/−	3	−	110(3)	Healthy
μ−/−	3	αCD4/αCD8	15.7 ± 0.6	AMD
μ+/+	3	−	110(3)	Healthy
μ+/+	3	αCD4/αCD8	110(3)	Healthy

*Mice were killed healthy or moribund on the indicated day. Moribund mice had ruffled fur, pale ear color, and hunched posture, and would be expected to die within 24 h according to previous experiments.
[‡]Standard deviation.
[§]No. of mice.
GI, gastrointestinal disease.

by the presence or absence of NK cells (17). The fact that this pathology was very different from the tumor induction described in nude mice indicated that B cells and/or extrathymically developing T cells might have an important role in PyV pathogenesis. To address this question, we infected mice having various T and/or B cell deficiencies with PyV (Table 1).

Mice that had a targeted disruption in the TCR α or β gene (αβ knockout mice) (24) survived the acute phase of PyV infection and did not show any symptoms of the AMD up until 3 mo after infection. Depletion of the TCR αβ knockout mice of NK cells in vivo by anti-NK1.1 antibodies did not influence this outcome. Mice deficient in both αβ and γδ T cells (22) also survived, regardless of the presence or absence of NK cells. Two of three αβ knockout mice and one of four NK-depleted αβ knockout mice had to be killed between day 28 and 69 after infection, because the animals had gastrointestinal obstruction or serious wasting associated with diarrhea. These symptoms were consistent with an ulcerative colitislike pathology that is known to affect this mutant mouse strain (32). Therefore, we consider the death of these animals not PyV related.

Mice deficient in functional B cells due to a targeted disruption of the Ig μ chain gene (μ−/−) (23) were also resistant to the PyV-induced acute myeloproliferation. However, when these μ−/− mice were depleted in vivo of CD4+ and CD8+ T cells by combined injections with anti-

CD4 and anti-CD8 antibodies, the animals (three of three) died of AMD by day 16, similarly to SCID mice. Mice with functional B cells from the same genetic background did not show any sign of PyV-induced disease, even when depleted of CD4+ and CD8+ T cells. These experiments indicate that although T cells can provide resistance against PyV infection, even in the absence of B cells, if functional CD4+ and CD8+ T cells are not present, B cells can mount an effective antiviral response as a second line of defense.

Prevention of PyV-induced AMD by Adoptive Transfer of B Cells into SCID Mice. To further investigate the role of different cell populations of the immune system in the resistance to PyV infection, adoptive transfer experiments were performed (Table 2). Spleen cells were prepared from uninfected, immunocompetent BALB/c mice. This cell population was used directly for adoptive transfer, or was first depleted of T cells in vitro by lysing the cells with anti-Thy-1 antibody and complement. The success of the depletion was monitored by FACS® staining. Splenocytes from αβ T cell knockout mice, B cell knockout mice, and B cell knockout mice after T cell depletion were also used for adoptive transfer into SCID mice. Adoptive transfer of the total spleen cell population (T and B cells) protected SCID mice not only from AMD, but from later tumor induction as well. All mice receiving B cells (Thy-1–depleted splenocytes, or spleen cells from αβ knockout mice) were protected from the AMD, but in a fraction of these ani-

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Table 2. *Protection of C.B17 SCID Mice from PyV-induced Acute Disease by Adoptive Cell Transfer*

	No. of mice	Source of cells transferred	Depletion (in vitro)	Cell No. given	Day Killed*	Disease
Exp. 1	2	C.B17 spleen	αThy-1	2.5×10^7	96	Tumor (1)[‡]
						Healthy (1)
Exp. 2	3	C.B17 spleen	—	10^8	105	Healthy (3)
	3	C.B17 spleen	αThy-1	5×10^7	90	Hind leg Paralyzed (1)
						Healthy (2)
	3	TCR αβ−/−	—	ND[§]	68	Tumors (2)
					68	Healthy (1)
Exp. 3	4	μ−/− spleen	αThy-1	1.1×10^6	13.2	AMD (4)
	4	μ+/+ spleen	αThy-1	3×10^7	82	Tumors (2)
						Healthy (2)

*Mice were killed healthy or moribund on the indicated day.
[‡]No. of mice.
[§]Cell number was not determined, cells prepared from three αβ knockout mouse spleens were injected into three recipient SCID mice.

mals, PyV infection induced multiple mammary tumors. This finding is consistent with published data showing a high frequency of mammary tumors in female BALB/c nude mice (21). Four out of four SCID mice that received adoptive transfer of T cell–depleted splenocytes from B cell knockout mice died by day 14 with symptoms of AMD, indicating the essential role of B cells in protection when T cells were absent.

PyV Replication in SCID Mice Reconstituted with B or B and T Cells. Whereas in immunocompetent or nude mice the PyV replication is not detectable in most major organs by 2 wk after infection, in SCID mice high levels of virus replication have been observed at this time in all organs tested (17, 20). We asked whether the protection of SCID mice from the AMD by B cells was associated with a major decrease in the amount of the virus in different organs. SCID mice, SCID mice adoptively transferred with B cells (Thy-1–depleted splenocytes) or with B and T cells (undepleted spleen cells) were infected with PyV and killed at days 3, 7, and 14 after infection. The presence of viral genome in DNA samples prepared from spleen, kidney, liver, and lung tissues of the animals was analyzed by Southern blots, using cloned, ^{32}P-labeled PyV A2 genome as the hybridization probe. At day 3, all three groups of mice had viral signal in the spleen (Fig. 1 *A*). At this time DNA samples from other organs (kidney, lung, and liver) did not show the presence of viral genome, even after a long exposure of the blots (kidney and lung not shown). At day 7, strong viral bands could be observed in all four organs in SCID mice, a finding consistent with our previous results (17). In SCID mice adoptively transferred with B cells alone, or with B and T cells, very little or no virus-specific DNA was detected in any of the organs tested. Even in the spleen the viral signal, which could be observed at day 3, was largely diminished by day 7 (Fig. 1 *B*). At day 14, whereas PyV could be de-

tected in all four organs in SCID mice, the virus signal was absent from SCID mice receiving B cells or T and B cells (Fig. 1 *C* and Fig. 2 *B*). The fact that we could not observe significant differences in the efficiency of PyV clearance in the spleen, kidneys, liver, or lung by B cells alone or by T and B cells together at day 7 and 14 after infections indi-
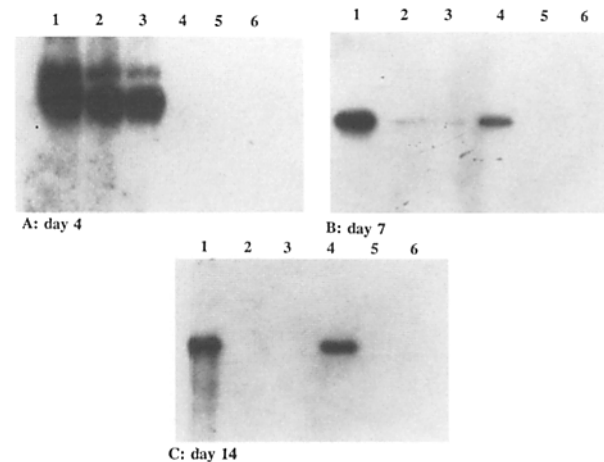

A: day 4

B: day 7

C: day 14

Figure 1. Effect of adoptive transfer of B and T cells on PyV replication in spleen and liver of C.B17 SCID mice. Southern blots of DNA samples (5 μg) prepared from organs of SCID mice, SCID mice reconstituted with Thy-1–depleted BALB/c spleen cells (SCID+B cells), and SCID mice reconstituted with spleen cells of BALB/c mice (SCID+ T+B cells) taken at different times after infection. The blots were probed with ^{32}P-labeled cloned PyV genome. (*A*) Uncut DNA of spleens (lanes 1–3) and livers (lanes 4–6) at day 4 after infection. SCID mice (lanes 1 and 4), SCID+B cells (lanes 2 and 5), and SCID+T+B cells (lanes 3 and 6). (*B*) Day 7 postinfection EcoRI-cut DNA from spleens (lanes 1–3) and livers (lanes 4–6) of SCID mice (lanes 1 and 4), SCID+B cells (lanes 2 and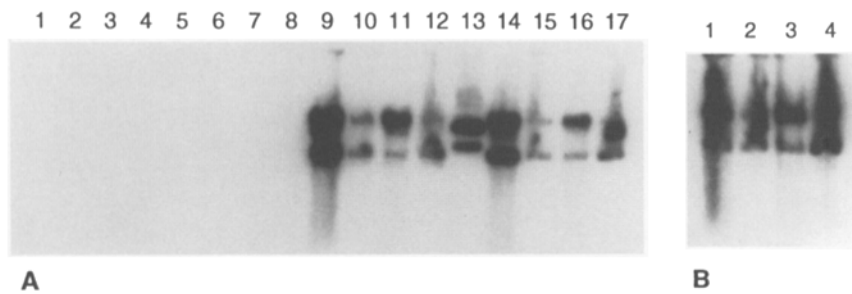 5), SCID+T+B cells (lanes 3 and 6). (*C*) Day 14 postinfection EcoRI-cut DNA from spleens (lanes 1–3) and livers (lanes 4–6) of SCID mice (lanes 1 and 4), SCID+B cells (lanes 2 and 5), SCID+T+B cells (lanes 3 and 6).

Figure 2. Transfer of serum from PyV-infected αβ knockout mice into C.B17 SCID mice causes major delay in PyV replication. (*A*) PyV-infected SCID mice treated with serum of PyV-infected αβ knockout mice; Southern blot of DNA samples (1 μg/lane) from spleen (lanes *1*, *5*, *9*, and *15*), kidney (*2*, *6*, *10*, and *16*), liver (*3*, *7*, *11*, and *17*), and lung (*4*, *8*, *12*, and *18*). Lanes *1–4*: day 4 after infection, lanes *5–8*: day 13, lanes *9–13* and *15–18*: day 32. Lane *14*: 0.1 ng pAT153-PyV A2 plasmid DNA. (*B*) PyV-infected SCID mouse. Day 13 after infection. Lane *1*: spleen, lane *2*: kidney, lane *3*: liver, and lane *4*: lung. Probe: ³²P-labeled, cloned PyV A2 genome.

cates that B cells could control PyV replication in these organs in the absence of T cells.

Protection Against AMD Is Mediated by Serum. We tested whether serum from PyV-infected αβ knockout mice could transfer protection to SCID mice against PyV-induced AMD. αβ knockout mice and C57BL/6 mice were infected with PyV; 7 d after infection, the mice were bled and serum was harvested. C.B17 SCID mice were then injected with the sera 1 d before PyV infection. Sera from normal, uninfected mice were used as negative controls. Sera from PyV-infected C57BL/6 mice or αβ knockout mice prolonged the survival of the SCID mice, regardless of the route of injection (i.p. or i.v.) (Table 3). Sera from uninfected mice did not have any effect on survival. The mice that received "PyV-immune" sera died between day 37 and 48 after infection with the pathological signs of AMD. This indicates that a single injection of serum could not completely prevent but could delay the lethal outcome by 3–4 wk; the same 3–4-wk delay in the onset of AMD was observed when serum harvested at day 14 after PyV infection of αβ knockout mice was used (Table 4). The prolonged survival was accompanied by a delay in the kinetics of virus replication (Fig. 2). In SCID mice treated with day 7 serum of PyV-infected αβ T cell–deficient mice, no viral DNA could be detected by Southern blots at day 4 and 14

after PyV infection, although by this time in untreated SCID mice, high levels of PyV replication could be observed in all organs tested. However, by day 32, the PyV genome became readily detectable in samples of spleen, kidney, liver, and lung DNA from the PyV-immune serum-treated SCID mice (data from two individual mice are shown in Fig. 2).

Serum of PyV-infected T Cell–deficient Mice Contains Virus-specific IgM and IgG. To determine whether serum derived from PyV-infected αβ knockout mice at day 14 after infection contains PyV-specific IgM or IgG antibodies, indirect immunofluorescent staining experiments were performed. 3T3 cells infected with PyV for 3 d were reacted with a 1:20 dilution of the serum, followed by staining with FITC-conjugated murine μ chain– or murine γ chain–specific second antibody. Bright nuclear fluorescent staining of the PyV-infected cells could be observed with the γ chain–specific reagent (Fig. 3 *A*). This staining was specific, because PyV-infected cells exposed to non-PyV–immune serum and the anti-γ-FITC antibody exhibited only diffuse, cytoplasmic background fluorescence (Fig. 3 *B*.). Using PyV-immune αβ knockout serum with FITC-antimurine μ antibody, a virus-specific nuclear fluorescent signal was also observed, indicating that T cell–deficient mice mount virus-specific IgM and IgG responses against PyV. Immunofluorescent

Table 3. *Effect of Serum Transfer on the Survival Time of PyV-infected C.B17 SCID Mice*

	No. of mice	Source of serum transferred	Serum dose	Route of injection	Killed* (day)	Disease
Exp. 1	3	Uninfected C57BL	500 ml	i.p.	13.3 ± 0.6‡	AMD
	3	PyV-infected C57BL§	500 ml	i.p.	45 (3)‖	AMD
Exp. 2	1	Uninfected C57BL	100 + 250 μl	i.v. + i.p.	15 (1)	AMD
	2	PyV-infected TCR αβ −/−§	100 + 250 μl	i.v. + i.p.	40 (2)	AMD
Exp. 3	2	Uninfected C57BL	200 μl	i.v.	14 (2)	AMD
	2	PyV-infected C57BL§	200 μl	i.v.	37 (2)	AMD
	2	PyV-infected TCR αβ−/−§	200 μl	i.v.	48 (2)	AMD

*Mice were killed moribund on the indicated day. Moribund mice had ruffled fur, pale ear color, and hunched posture, and would be expected to die within 24 h according to our previous experiments.
‡Standard deviation.
§Serum was harvested at day 7 after infection.
‖Number of mice.

Table 4. *Ig Fractions of PyV-infected αβ Knockout Mouse Serum Prolong the Survival of PyV-infected C.B17 SCID Mice*

	No. of Mice*	Serum given (i.v.)	Volume	Titer[‡]	Time of death[§]
			μl		*d*
Exp. 1	2	None	—	—	16,17
	2	Naive αβ knockout serum	150	ND	15,18
	2	Naive normal mouse serum	100	ND	16,17
	2	PyV-infected, αβ knockout serum[‖]	100	ND	39 +[¶]
	2	Peak I[**]	200	<1:20	20,21
	2	Peak I[**]	450	<1:20[‡‡]	21,21
	2	Peak II[§§]	200	1:20	19,21
Exp. 2	2	None	—	—	13,13
	2	PBS	100	—	15,17
	2	PyV-infected, αβ knockout serum[‖]	100	1:800	39,48
	2	IgG fr.4[‖‖‖]	100	1:200	33,41
	2	IgG fr.5[‖‖‖]	150	1:80	36,40

*Mice were injected with 10[5] TCID$_{50}$ of PyV strain A2 i.p. on the day of i.v. serum injections.

[‡]Titer defined as the highest dilution of serum resulting in positive immunofluorescence of PyV-infected 3T3 cells with an IgG-specific FITC-labeled second antibody.

[§]Mice were killed moribund on the indicated day (see footnote to Tables 1 and 3).

[‖]Serum was harvested on day 14 after infection.

[¶]One mouse died on day 39, the other was killed with no symptoms.

[**]Peak I chromatography fraction contained both IgM and IgG.

[‡‡]Peak I fraction showed positive IgG-specific fluorescence undiluted, but in 1:20 dilution was negative.

[§§]Peak II chromatography fraction contained only IgG.

[‖‖‖]IgG-containing fractions of αβ knockout mouse serum harvested 14 d after PyV infection were obtained by chromatography on a protein A–Sepharose column (see Materials and Methods).

staining of PyV-infected 3T3 cells with serum of PyV-infected αβ knockout mice (1:20 dilution) and FITC-labeled second antibodies specific for subclasses of IgG revealed that the serum of PyV-infected αβ knockout mice contained predominantly IgG2a, as IgG1-, IgG2b-, and IgG3-specific staining was not detectable. However, bright nuclear fluorescence was observed with the anti-IgG2a-FITC antibody. Sera from PyV-infected, NK-depleted αβγδ knockout mice harvested 2 wk after infection also contained levels of IgG2a comparable to those of the PyV-infected αβ knockout mouse serum. Staining with a 1:100 dilution of this sera and IgG2a-specific FITC-labeled antibodies resulted in bright nuclear fluorescence of PyV-infected cells (not shown).

To test whether purified IgM or IgG fractions of PyV-infected αβ knockout mice can protect SCID mice against the acute PyV-induced disease, the serum was fractionated by chromatography on a Sephacryl 300 (Pharmacia Biotech, Uppsala, Sweden) column. The first protein peak eluted from the column was enriched for IgM, but contained IgG as well. The second protein peak contained IgG, but no detectable IgM. SCID mice injected with 200 μl of either fraction survived PyV infection significantly longer than SCID mice treated with sera of naive knockout mice before infection (Table 4, Exp. 1). In a second experiment, high titer antiviral IgG was purified by protein A–Sepharose

chromatography from the serum of αβ knockout mice harvested on day 14 after infection. C.B17 SCID mice were injected intravenously with unfractionated PyV-infected αβ knockout serum (titer 1:800) and the peak IgG fractions fr.4 and fr.5 (titer 1:200 and 1:80, respectively) (Table 4, Exp. 2). Whereas all the mice without serum or IgG treatment died by day 17 (mean survival time 14.5 ± 1.9), mice that received the purified IgG fractions from PyV-infected αβ knockout mouse serum survived 19–27 d longer. Furthermore, this survival time was very similar to that of mice injected with unfractionated PyV-infected αβ knockout mouse serum, indicating that IgG produced in the absence of T cells expressing αβ TCR provides protection against the lethal outcome of PyV infection (Table 4).

Discussion

We show here that B cells in the absence of T cells protect mice from PyV-induced lethal AMD. This protection is mediated by the induction of PyV-specific IgM and IgG antibodies, predominantly IgG2a, produced without any T cell help. These antibodies then clear PyV in different organs of the PyV-infected mice. To our knowledge, this is the first example of a completely T cell–independent antibody response effectively reducing virus load ("clearance")
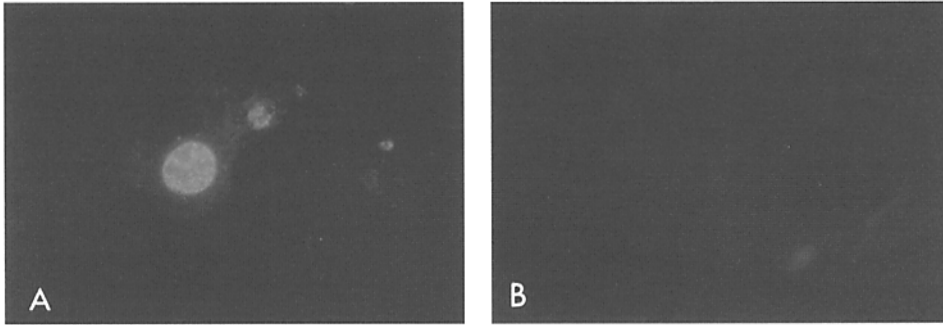
Figure 3. Serum from PyV-infected αβ knockout mice contains PyV-specific IgG. Staining of PyV-infected cells with (A) 1:20 dilution of serum from PyV-infected αβ knockout mice and FITC-labeled antibody specific for mouse IgG γ chain. Strong nuclear fluorescence of PyV-infected cells indicates the presence of PyV-specific IgG in the serum. (B) 1:20 dilution of serum from uninfected mice and FITC-labeled mouse IgG γ chain–specific antibody. Only weak cytoplasmic background staining can be observed.

and thereby dramatically changing the outcome of a virus infection (from 100% lethality by day 16 to 100% survival).

It is well known that virus-specific antibodies can prevent infection or reinfection by neutralization (33), and several studies have indicated that antibodies are also capable of mediating virus clearance in vivo. Examples of this are reovirus and Sindbis virus clearance in the central nervous system of neonatal or SCID mice by the injection of monoclonal antibodies (8, 34), or influenza virus clearance by transfer of monoclonal antibodies into SCID mice (35). However, the generation of an efficient, high-affinity antibody response and the switching from IgM to IgG or IgA production usually requires T cell help (4, 5). It is very interesting therefore, that PyV is an inducer of a protective antibody response in T cell–deficient mice. In PyV-infected SCID mice, the virus replicates to high levels, and the high level of virus antigens might be an important requirement for triggering T cell–independent antibody production. This is also suggested by a recent publication by Freer et al. (36), which reported induction of VSV-specific IgG in nude mice. In that system, the T cell dependence of both IgG and, to a lesser degree, IgM, seemed to be dependent on the antigen dose, and only at very high antigen levels of UV-inactivated VSV was an "inefficient but significant" switch to IgG synthesis observed. However, since nude mice are not completely free of T cells, the possibility of helper factors produced by the remaining T cells was not excluded in those experiments.

It is interesting to speculate which factors might contribute to the efficient switch from IgM to IgG production in the absence of any T cell help (and even in the absence of NK cells) in the case of PyV infection. One factor in overcoming the lack of usual helper functions might be a characteristic feature of the antigen. The major capsid glycoprotein PyV, VP1, is repeated periodically 360 times (in 72 pentamers) in the virion capsid structure (37, 38). T cell–independent antigens typically consist of multiple repeating epitopes that allow for extensive cross-linking of the antigen receptor on the B cell surface. Most T cell–independent antigens are polysaccharides, but polymeric protein structures with repetitive epitopes, such as bacterial flagellin, can be T cell–independent antigens as well (39, 40). Therefore, the structural periodicity of the PyV virion capsids might be responsible, at least in part, for the ability to trigger T cell–independent antibody responses. This idea is supported by a report by Bachmann et al. (41), which describes that transgenic mice tolerant to nonrepetitive VSV glycoprotein antigen produced antibodies in response to the highly organized, repetitive form of the same antigen represented by formalin-inactivated virions. Therefore, the organization and density of antigenic epitopes had an important role in determining B cell induction. However, since many viruses have capsids with similar (icosahedral) repetitive structures, it is surprising that T cell–independent antibody responses influencing the pathogenesis of other virus infections have not been reported so far. The newly available mouse strains with complete and selective T cell deficiencies will be useful for determining how widespread this phenomenon is. Other factors in PyV-infected T cell–deficient mice, such as the production of cytokines, may also contribute to the induction of a strong T cell–independent antibody response.

Because the serum from PyV-infected αβ knockout mice has some neutralizing activity in vitro (preliminary data, not shown), neutralization of PyV would be an obvious mechanism of action of the antiviral antibodies. However, the protective effects of antibodies in vivo do not necessarily correlate with their in vitro neutralizing abilities (42–44). Both neutralizing and nonneutralizing antibodies can clear Sindbis virus in the infected central nervous system. In this case, viral protein synthesis and virus assembly are inhibited by the antibodies, which bind to the membrane of the virus-infected cells (8). In LCMV infection, reduction of viral titers in tissues is mediated by some, but not all neutralizing antibodies. It is interesting to note that antibodies with IgG2a isotype are the best at protecting against LCMV (43), and that this isotype was shown to be a major component in the sera of PyV-infected, T cell–deficient mice. IgG2a is known to be predominantly produced in response to virus infections in general, and to PyV infection in particular, in immunocompetent mice (45). It is surprising, however, that this happens even in the absence of T cells after PyV infection.

In summary, our results show that PyV can behave as a T cell–independent antigen and provide the first unambigous example we know of a virus inducing a physiologically relevant, T cell–independent antibody response that can control the infection.

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