3. G. W. Van Hoesen, R. J. Morecraft, B. A. Vogt, in (*5*), pp. 249–284.
4. R. B. Morecraft and G. W. Van Hoesen, *J. Comp. Neurol.* **322**, 471 (1992); S. Q. He, R. P. Dum, P. L. Strick, *J. Neurosci.* **15**, 3284 (1995).
5. B. A. Vogt and M. Gabriel, Eds., *Neurobiology of Cingulate Cortex and Limbic Thalamus* (Birkhäuser, Boston, 1993).
6. A. Partiot, J. Grafman, N. Sadato, J. Wachs, M. Hallett, *Neuroreport* **6**, 1397 (1995).
7. N. Picard and P. L. Strick, *Cereb. Cortex* **6**, 342 (1996).
8. The animals were cared for in accordance with NIH's *Guide for Care and Use of Laboratory Animals*, and *Guidelines for Institutional Animal Care and Use* published by Tohoku University School of Medicine.
9. Behavioral task: When the monkey held the handle in a neutral position for 2.8 to 7.8 s, an LED (light-emitting diode) was illuminated as a signal to start the correct movement. Initially, the subject had to guess which of the two choices was correct. Performing the correct movement was rewarded with a drop of fruit juice, and the correct movement remained unchanged in a block of trials, so that the monkey was required to keep selecting the same movement. The amount of the reward remained constant (0.1 ml) for four to 12 successive trials, unless the subject made a mistake and selected the wrong movement, in which case an audible warning tone replaced the reward. Subsequently, the amount of the reward decreased by 30% for each correct trial. At this stage, monkeys were free to select the alternate movement. They usually did so after the first to the third decrement (30 to 65.7% decrease in reward). If they did, the alternate movement was then defined as the correct movement, the reward reverted to the full amount, and a new series of constant-reward trials began, with the redefined correct movement.
10. K. Shima *et al.*, *J. Neurophysiol.* **65**, 188 (1991); J. Tanji, *Neurosci. Res.* **19**, 251 (1994).
11. After completion of recording cellular activity, recording sites were marked with microlesions (by passing small currents through recording electrodes). Thereafter, the primary motor cortex was carefully mapped with intracortical microstimulation. A chemical tracer, wheat-germ agglutinin–horseradish peroxidase (WGA-HRP, 0.2 μl), was injected into four sites within both the proximal and distal forelimb areas of the primary motor cortex. In the recording sites in the CMAs, HRP-positive cells were found with a standard technique (*10*). Because we found no spread of injected WGA-HRP into the face area, we concluded that the portions of CMAr and CMAc from where we recorded cellular activity coincided with the areas that projected to arm representation areas of the primary motor cortex.
12. Intracortical microstimulation was delivered with 20 to 40 pulses of 0.2-ms duration at 333 Hz at 20 to 50 μA. Responses of cells to somatosensory stimulation (responses to joint manipulations or stroking the skin) also helped to find that the recording sites were forelimb representation areas and not face or neck areas.
13. Neuronal activity was quantitatively analyzed after constructing a peri-event histogram by summing the data from at least 10 trials for each condition. We defined the activity as task-related when the number of discharges in at least three successive 20-ms bins of the peri-event histogram during the four task periods deviated from the mean value during a control period by more than 2 SDs. The task periods included preparatory (from the time the handle was held in the central position to the trigger signal), premovement (from the trigger signal to the onset of movement), postmovement (from the onset of movement to delivery of the reward, 400 ms after the execution of the movement), and reward (500 ms after reward delivery). The control period was the last 500 ms of the intertrial interval. We performed analysis of variance (ANOVA) to test whether the activity in the reduced reward condition differed from that in the ordinary reward condition for the data obtained on a trial-by-trial basis.
14. We performed ANOVA to test this difference.
15. This finding suggests that the activity of these selective cells influences a motor decision process (facilitating selection of one movement and suppressing the other), rather than reflecting an affective aspect of the reward.
16. Y. Matsuzaka and J. Tanji, *J. Neurophysiol.* **76**, 2327 (1996).
17. The neurons were recorded from both the proximal (arm) and distal (hand) representation areas in the primary motor cortex, defined by the effects of intracortical microstimulation. In addition to this study on the 114 neurons in these areas, we also examined neuronal responses in the neck, back, and shoulder representation areas. In these areas, we did not observe the selective responses appearing after the reduced reward. We also analyzed extensively the activity in the neck and back muscles (with electromyogram) and found no responses appearing selectively after the reduced reward.
18. After completing the cellular recording sessions, we inserted small-caliber (300 μm) injection cannulae bilaterally into the CMAs and injected a small amount (2 to 4 μl, 1 to 10 μg/μl, 1 μl in 10 min) of muscimol to evaluate the effects of temporary deactivation of each area. The least effective concentration was 5 μg/μl.
19. The reaction time (defined as the interval between the beginning of the "start" signal and the movement onset) and movement time (the interval from the movement onset to the attainment of the required motor-target) during performance of the motor task were always analyzed. When the movement alteration was cued with the tone signal, neither values was found to be significantly different when compared before and after the muscimol injection ($P > 0.1$ by Mann-Whitney U test). This means that the animals' ability to execute the movements per se was not much influenced by the muscimol application. However, after the muscimol injection, the reaction time was lengthened ($P < 0.01$) when the animal selected the alternate movement in response to the reduced reward, although the movement time was not lengthened ($P > 0.05$).
20. C. D. Frith, K. Friston, P. F. Liddle, R. S. J. Frackowiak, *Proc. R. Soc. London Ser. B* **244**, 241 (1991); E. D. Playford, I. H. Jenkins, R. E. Passingham, U. Nutt, R. S. J. Frackowiak, *Ann. Neurol.* **32**, 151 (1992); T. Paus, M. Petrides, A. C. Evans, E. Meyer, *J. Neurophysiol.* **70**, 453 (1993); I. H. Jenkins, D. J. Brooks, P. D. Nixon, R. S. J. Frackowiak, R. E. Passingham, *J. Neurosci.* **14**, 3775 (1994).
21. D. G. Amaral and J. L. Price, *J. Comp. Neurol.* **230**, 465 (1984); C. Baleydier and F. Mauguiere, *Brain* **103**, 525 (1980); B. A. Vogt and D. N. Pandya, *J. Comp. Neurol.* **262**, 271 (1987).
22. P. S. Goldman-Rakic, *Neuron* **14**, 477 (1995).
23. Supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Japanese Ministry of Education, Science, Sports and Culture.

17 August 1998; accepted 13 October 1998

Control of Neonatal Tolerance to Tissue Antigens by Peripheral T Cell Trafficking

Judith Alferink, Anna Tafuri,* Dietmar Vestweber, Rupert Hallmann, Günter J. Hämmerling, Bernd Arnold†

Self tolerance is acquired by the developing immune system. As reported here, particular properties of the neonatal tissue contribute to this process. Neonatal skin, but not adult skin, was accessible for naïve CD8 T cells. In mouse bone marrow chimeras generated at different ages, recent thymic emigrants were tolerized to a skin-expressed major histocompatibility complex class I antigen only during a neonatal period but not during adulthood. Blockade of T cell migration neonatally prevented tolerance induction. Thus, T cell trafficking through nonlymphoid tissues in the neonate is crucial for the establishment of self tolerance to sessile, skin-expressed antigens.

Differences in tolerance induction during the neonatal and adult periods of life have fascinated immunologists since the pioneering work of Billingham, Brent, and Medawar (*1*). Neonatal mice, in contrast to adults, develop lifelong tolerance to allogeneic skin grafts when exposed to allogeneic cells of the same donor strain; hence, self tolerance is actively acquired. The newborn immune system can also mount an immune response when challenged (*2*). Thus, there appear to be quantitative but not qualitative differences among the cells generating an immune response (*2*).

Although these investigations have focused on systems in which mobile antigen-presenting cells pick up antigen and carry it to lymphoid organs for T cell recognition, the role of differential T cell migration in tolerance induction to sessile self antigens expressed exclusively on extrathymic tissues is undefined. Large-scale trafficking of virgin T cells through extralymphoid tissues has been observed in fetal sheep, in contrast to the restricted circulation in the adult animal (*3*). To test whether differential T cell migration through neonatal versus adult tissue would influence tolerance induction to tissue-specific self antigens, we used a transgenic mouse model expressing the major histocompatibility complex (MHC) class I antigen K^b under

J. Alferink, A. Tafuri, G. J. Hämmerling, B. Arnold, Tumor Immunology Program, German Cancer Research Center, 69120 Heidelberg, Germany. D. Vestweber, Institute of Cell Biology, University of Münster, 48149 Münster, Germany. R. Hallmann, Institute for Experimental Medicine, University of Erlangen-Nürnberg, 91054 Erlangen, Germany.

*Present address: Ontario Cancer Institute/Amgen Institute, Toronto, Ontario M5G 2C1, Canada.
†To whom correspondence should be addressed.

control of the constitutively expressed keratin IV promoter on skin keratinocytes. These 2.4KerIV-K^b mice exhibit peripheral tolerance characterized by the presence of K^b-specific T cells that do not reject K^b-positive grafts and hence are functionally tolerant (*4*).

We first investigated whether K^b expressed on keratinocytes in the adult mouse could induce T cell tolerance. The following bone marrow (BM) chimeras were established: Adult 2.4KerIV-K^b mice on the Rag-$2^{-/-}$ background (*5*) were reconstituted with T cell–depleted BM cells from K^b-specific T cell receptor (Des-TCR) transgenic mice (Des-TCR → 2.4KerIV-K^b.Rag-$2^{-/-}$). Efficient reconstitution visualized with the anticlonotypic antibody Désiré-1 (*6*) was obtained 2 to 3 months after transfer of Des-TCR BM cells (Fig. 1). Des-TCR → Rag-$2^{-/-}$ chimeras were included to test the K^b responsiveness of Des-TCR T cells in the absence of the K^b transgene. Tolerance was assessed by injecting chimeric mice with K^b-positive P815 tumor cells (*4*) 2 to 3 months after reconstitution. P815.K^b tumors are rejected by single-transgenic Des-TCR mice and accepted by tolerant double-transgenic Des-TCRx2.4KerIV-K^b mice (*7*). The chimeric mice that had been reconstituted as adults rejected the P815.K^b tumor (Table 1, group A) and were therefore not tolerant. This was confirmed in another assay system. After grafting Des-TCR → 2.4KerIV-K^b.Rag-$2^{-/-}$ chimeras with skin autografts from their tails onto their lateral thoracic walls, rejection became visible in all seven recipients after 14 days (*8*). These findings show that tolerance to the K^b self antigen is not induced when the antigen is expressed by mature peripheral tissue in the adult environment.

Is there a critical time window during early development for tolerization to the skin-expressed K^b antigen? We injected 2.4KerIV-K^b.Rag-$2^{-/-}$ mice with Des-TCR BM cells either at birth or at the age of 15 days (Fig. 1, B and C) (*5*) and assessed tolerance after 2 to 3 months. In the neonatal chimeras, Des-TCR T cells were already found in the peripheral blood on day 19 after reconstitution and rapidly increased afterward. Tolerance was observed in these chimeras (Table 1, group C). This result is consistent with the observation that K^b-specific tolerance is induced in Des-TCRx2.4KerIV-K^b mice expressing K^b on keratinocytes and the Des-TCR constitutively from birth. In contrast, 2.4KerIV-K^b.Rag-$2^{-/-}$ mice reconstituted at 15 days of age rejected the P815.K^b tumor cells (Table 1, group B). Thus, tolerance induction to skin-expressed K^b antigen appears to occur only during an early period after birth.

Support for this view was also derived from the following experimental approach: Elimination of Des-TCR T cells with the anticlonotypic antibody until day 15 after birth abolished tolerance induction in Des-TCRx2.4KerIV-K^b mice. Six of seven mice that had received the antibody treatment rejected P815.K^b tumor cells grafted as adults, compared to 1 of 18 that did not receive antibody (*9*). The Désiré antibody-treated mice displayed on day 18 less than 1% and on day 24 only 4 to 5% Des-TCR T cells in the peripheral blood. Thus, the reconstitution and the antibody treatment experiment indicate a time window of about 3 to 4 weeks after birth as a rough approximation for the tolerance-sensitive period.

Tolerance induction in the neonate, in contrast to that in the adult, could be attributable either to intrinsic properties of T cells leaving the neonatal versus adult thymus or to

Table 1. Tumor acceptance in BM chimeric mice. Recipients were reconstituted at various ages, with (group D) or without (groups A, B, and C) thymectomy and engraftment with a neonatal 2.4KerIV-K^b thymus. Mice in groups A, B, and C were injected subcutaneously with 10^5 P815.K^b tumor cells 10 to 14 weeks after reconstitution with Des-TCR BM cells (*5*). Mice in group D were thymectomized and grafted with a neonatal thymus from 2.4Ker-K^b mice at the age of 5 weeks, followed by reconstitution with Des-TCR BM cells; P815.K^b cells were injected subcutaneously 6 weeks later (*5*). Mice in group E were nonchimeric controls injected with P815.K^b cells at the age of 8 to 12 weeks.

Experimental group	Recipient mice	Age at reconstitution	Acceptance of P815.K^b tumor cells
A	2.4KerIV-K^b.Rag-$2^{-/-}$	5 weeks	3/15
	Rag-$2^{-/-}$	5 weeks	4/21
B	2.4KerIV-K^b.Rag-$2^{-/-}$	15 days	1/4
	Rag-$2^{-/-}$	15 days	0/4
C	2.4KerIV-K^b.Rag-$2^{-/-}$	24 hours	4/4
	Rag-$2^{-/-}$	24 hours	0/3
D	2.4KerIV-K^b.Rag-$2^{-/-}$	5 weeks	1/7
	Rag-$2^{-/-}$	5 weeks	0/4
E	Des-TCRx2.4KerIV-K^b	–	22/24
	Des-TCR	–	1/24

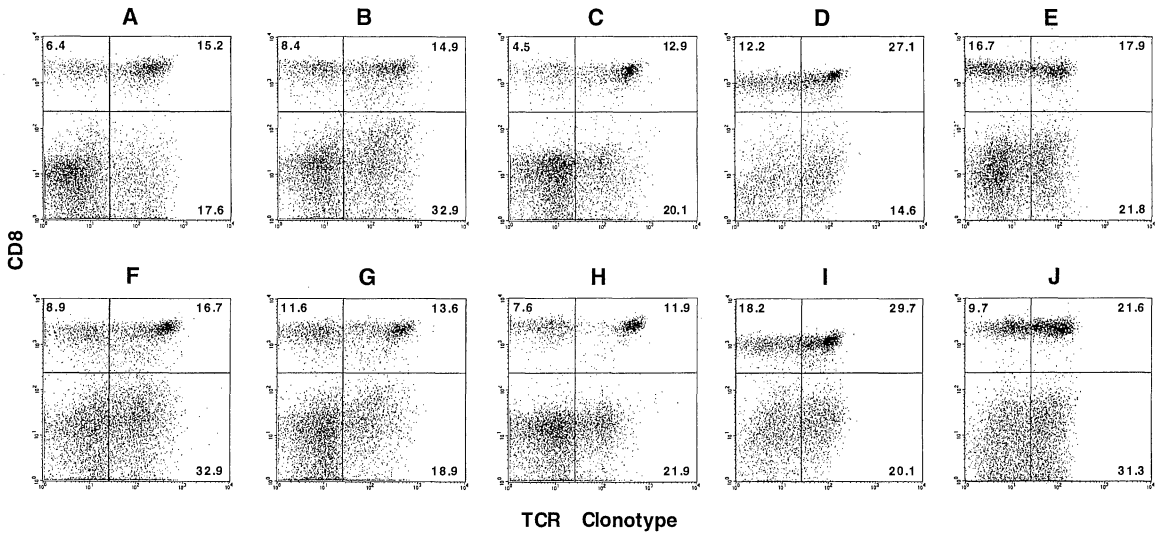

Fig. 1. T cell phenotype of 2.4KerIV-K^b.Rag-$2^{-/-}$ and Rag-$2^{-/-}$ mice 10 to 14 weeks after reconstitution with Des-TCR BM cells. (**A** to **C**) Two-color immunofluorescence of B cell–depleted splenocytes (*5*) from 5-week-old (A), 15-day-old (B), and neonatally (C) reconstituted Des-TCR → 2.4KerIV-K^b.Rag-$2^{-/-}$ BM chimeras stained for CD8 (vertical axis) and the clonotypic Des-TCR (horizontal axis). (**D**) Another group of 5-week-old thymectomized 2.4KerIV-K^b.Rag-$2^{-/-}$ mice were grafted with thymic lobes from neonatal 2.4KerIV-K^b transgenic mice before BM inoculation. (**E**) Untreated Des-TCRx2.4KerIV-K^b double-transgenic mice. (**F** to **I**) Corresponding control groups of Rag-$2^{-/-}$ mice were injected at 5 weeks (F), 15 days (G), on the first day after birth (H), or after thymectomy and grafting at the age of 5 weeks with a thymus from neonatal 2.4KerIV-K^b transgenic mice (I). (**J**) Des-TCR single-transgenic mice.

characteristics of the target tissue in the newborn mouse. To distinguish between these possibilities, we constructed thymus-BM chimeras by replacing the endogenous thymic rudiment of 5-week-old 2.4KerIV-K^b.Rag-$2^{-/-}$ or Rag-$2^{-/-}$ mice with the thymus of newborn 2.4KerIV-K^b animals, followed by reconstitution with Des-TCR BM cells (*5*) (Fig. 1, D and I). These chimeras rejected P815.K^b cells and were therefore not tolerant. Two conclusions can be drawn from these results. First, a neonatal 2.4KerIV-K^b thymus does not elicit K^b-specific tolerance in Rag-$2^{-/-}$ mice, thereby excluding the possibility of transient K^b expression in the neonatal thymus. Second, the age of the thymic tissue in which the T cells develop does not control their capacity to become tolerized by peripheral tissue antigens. Instead, the maturity of the peripheral tissue appears to be critical. Because the neonatal thymus graft was not irradiated, low numbers of recent thymic emigrants could already be detected in the periphery a few days after transplantation. Such T cells represent neonatal T cells emigrating during the tolerance-sensitive time window mentioned above.

Various characteristics of the neonatal tissue may account for tolerance induction in the neonatal Des-TCR → 2.4KerIV-K^b.Rag-$2^{-/-}$ chimeras. Differential K^b expression on keratinocytes of the neonatal and adult skin could be excluded by immunohistological analysis (*10*) and skin transplantation. The survival time of grafts from neonatal and adult 2.4KerIV-K^b.Rag-$2^{-/-}$ donors transplanted on Des-TCR mice was similar (*11*). Alternatively, an enhanced trafficking of naive T cells through peripheral tissues in the neonate may facilitate efficient encounter of the skin-expressed K^b antigen. Therefore, we investigated the homing pattern of lymphocytes in newborn and adult 2.4KerIV-K^b mice by injecting radiolabeled lymphocytes from Des-TCR mice (*12*). Four hours after transfer, 38% of ^{51}Cr-labeled T cells could be detected in the adult spleen, versus less than 1% in the neonatal spleen, similar to data in newborn rats (*13*). Instead, inoculated T cells accumulated in nonlymphoid tissues of neonatal recipients, such as lung and skin (Fig. 2). For example, 14% of injected lymphocytes entered the skin of neonatal 2.4KerIV-K^b mice, compared to only 2.3% in the skin of adult recipients; this ratio was independent of the number of injected cells. Intradermal trafficking of lymphocytes at the single-cell level, observed by means of vital fluorochromes (*14*), confirmed that neonatal skin is more accessible for naive T cells than adult skin.

The intradermal trafficking of naïve T cells in the newborn animal may have important implications for self-tolerance induction. Therefore, we asked whether blockade of T cell interaction with endothelia in the neonatal skin during the tolerance-sensitive phase would prevent tolerance induction. E- and P-selectin have been suggested to function as skin-selective adhesion molecules for T cells at sites of inflammation (*15*). In an orientation experiment, a single dose of antibodies to E- and P-selectin (20 μg), simultaneously given with ^{51}Cr-labeled T cells to neonatal 2.4KerIV-K^b mice, reduced homing to the skin by 39% after 12 hours but did not influence the migration pattern to other tissues. Therefore, newborn Des-TCRx2.4KerIV-K^b mice were treated for 15 days with antibodies and tested for tolerance at the age of 6 weeks (*16*). Most of these animals rejected K^b-positive tumor grafts, indicating that tolerance was abrogated, whereas newborn mice that were injected with control antibodies or received no injection accepted the P815.K^b tumor (Table 2) (*17, 18*). There was no sign of an impaired thymic maturation caused by the selectin monoclonal antibody (mAb) treatment, because the T cell phenotype was unchanged (*10*) and the respective T cells were functionally active in rejecting the P815.K^b tumor (Table 2). Thus, blocking T cell migration to the neonatal skin prevented tolerance induction to K^b expressed on keratinocytes.

Because recognition of cutaneous antigens by naïve CD8 T cells is apparently limited to the perinatal period, maintenance of self-protection in the adult must be controlled by alternative mechanisms, as naïve T cells continue to leave the thymus in adult life. Further studies will be required to determine whether regulation as described in various animal models (*19*) or other processes might account for the inability of the naïve anti-K^b T cells in the adult Des-TCRx2.4KerIV-K^b mice to reject grafts. Recent reports indicate that self antigens expressed by intact peripheral organs may be presented to CD8 T cells in the draining lymph nodes after uptake by dendritic or similar cells via an exogenous class I–restricted pathway. However, this phenomenon of cross-presentation was only observed when the tissue antigen was expressed in large amounts in the kidney and pancreas (*20*). In the present mouse model, however, we used a "foreign" MHC class I antigen and a corresponding T cell receptor recognizing only the intact K^b antigen but not a processed form of that antigen. Hence, our results exclude the possibility that antigen-presenting cells of the skin pick up the K^b antigen and present it in the neonate but not in the adult animal. The results indicate that the tolerance induction described here is dependent on migration of T cells into the skin. It is therefore different from cross-presentation and may represent a mechanism for tolerance induction to cutaneous antigens in situations where the expression of tissue antigens is too low for cross-presentation.

Table 2. Effect of E- and P-selectin mAbs on K^b-specific tolerance induction (*16*).

Recipient mice	Treatment	Acceptance of P815.K^b tumor cells
Des-TCRx2.4KerIV-K^b	mAbs to selectins	3/10
Des-TCRx2.4KerIV-K^b	Control Ab	3/3
Des-TCRx2.4KerIV-K^b	None	17/18

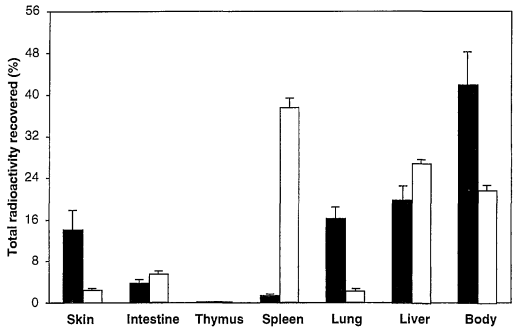

Fig. 2. Enhanced migration of Des-TCR T cells into the skin of newborn 2.4KerIV-K^b mice (solid bars) versus 5-week-old 2.4KerIV-K^b mice (open bars). ^{51}Cr-labeled B cell–depleted spleen and lymph node cells from Des-TCR mice were injected intravenously into six or seven mice per group, and various organs and remaining body were tested 4 hours after transfer (*12*). Data are shown as percentage of total counts per minute (measured per mouse) and are representative of three experiments.

References and Notes

1. R. E. Billingham, L. Brent, P. B. Medawar, *Nature* **172**, 603 (1953).
2. D. Abramowicz *et al.*, *Eur. J. Immunol.* **20**, 1647 (1990); J. W. Streilein, *Transplantation* **52**, 1 (1991); P.-F. Piguet, C. Irle, E. Kollatte, P. Vassalli, *J. Exp. Med.* **154**, 581 (1981); C. Martin, M. Belo, N. M. Le Douarin, C. Corbel, *Int. Immunol.* **6**, 795 (1994); P. J. Wood, P. G. Strome, J. W. Streilein, *J. Immunol.* **138**, 3661 (1987); T. Forsthuber, H. C. Yip, P. V. Lehmann, *Science* **271**, 1728 (1996); J. P. Ridge, E. J. Fuchs, P. Matzinger, *ibid.*, p. 1723; M. Sarzotti, D. S. Robbins, P. M. Hoffman, *ibid.*, p. 1726.
3. W. G. Kimpton, E. A. Washington, R. N. P. Cahill, *Int. Immunol.* **7**, 1567 (1995); C. R. Mackay, W. L. Marston, L. Dudler, *J. Exp. Med.* **171**, 801 (1990); R. J. Brezinschek, P. E. Lipsky, P. Galea, R. Vita, N. Oppenheimer-Marks, *J. Immunol.* **154**, 3062 (1995).
4. J. Alferink, B. Schittek, G. Schönrich, G. J. Hämmerling, B. Arnold, *Int. Immunol.* **7**, 331 (1995); G. Schönrich *et al.*, *ibid.* **4**, 581 (1992).
5. Chimeras were generated by intravenous inoculation of T cell–depleted BM cells from Des-TCR mice into nonirradiated 2.4KerIV-K^b.Rag-$2^{-/-}$ mice at different ages. For neonatal mice, 2×10^5 cells were injected into the anterior facial vein; 15-day-old and adult recipients were injected with 10^6 cells and 2×10^6 cells, respectively, in the tail vein. Phenotypic analysis of spleen and lymph node cells was performed with

mAbs specific for the clonotypic Des-TCR (Désiré-1) (*6*) and for mouse CD8 (53-6.7, Pharmingen).
6. C. Hua, C. Boyer, M. Buferne, A.-M. Schmitt-Verhulst, *J. Immunol.* **136**, 1937 (1986).
7. Des-TCRx2.4KerIV-K^b and Des-TCR mice have been described (*4*). All mice were kept under specific pathogen–free conditions at the central animal facility of the German Cancer Research Center. Tumor growth was evaluated as described (*4*).
8. Des-TCR → 2.4KerIV-K^b.Rag-$2^{-/-}$ chimeras were grafted under anesthesia on their lateral thoracic walls with skin autografts from their tails. Chronic rejection accompanied by hair loss of the skin autografts and contracted scar formation started after 14 days. After 4 weeks, a second skin transplant was rejected by the majority of chimeras (median survival time, 15 days). No autoimmune response against the endogenous K^b transgene–expressing keratinocytes could be seen by immunofluorescence histology, as judged by the absence of infiltrating Des-TCR T cells in the thoracic wall skin from the grafted and contralateral side.
9. Neonatal Des-TCRx2.4KerIV-K^b mice were injected every third day with 50 μg of Désiré-1 mAb during the first 15 days after birth, which reduced the Des-TCR T cells to less than 1%. Injection of P815.K^b was performed at the age of 6 weeks.
10. J. Alferink, A. Tafuri, D. Vestweber, R. Hallmann, G. J. Hämmerling, B. Arnold, data not shown.
11. Sixteen Des-TCR mice grafted on their lateral thoracic walls with skin grafts from either neonatal or 5-week-old 2.4KerIV-K^b.Rag-$2^{-/-}$ mice (eight mice per group) rejected the transplanted skin (median survival time, 24 days).
12. B cell–depleted spleen and lymph node cells from Des-TCR mice were ^{51}Cr-labeled and injected intravenously into groups of at least six neonatal (2×10^5 cells) and adult 2.4KerIV-K^b (2×10^6 cells) recipients. After whole-body perfusion, the distribution of radioactivity in various organs was determined in a γ-counter. Radiolabeled cells accumulated in neonatal versus adult skin in ratios of 8.41:1, 6.22:1, and 10:1 in three independent experiments.
13. T. E. Halstead and J. G. Hall, *Transplantation* **14**, 339 (1972).
14. B cell–depleted spleen and lymph node cells (10^6) from Des-TCR mice were labeled with 5,6-carboxy-succinimidyl fluorescein-ester (CFSE, 0.5 μm/ml; Molecular Probes) at 37°C for 15 min and injected into adult or neonatal 2.4KerIV-K^b mice. After 4 hours, whole-body perfusion was performed and the number of CFSE-labeled cells was determined by immunohistological analysis of the skin. CFSE-positive cells were enriched in neonatal skin and were only rarely visible in adult skin.
15. F. Austrup *et al.*, *Nature* **385**, 81 (1997); U. Henseleit *et al.*, *J. Pathol.* **180**, 317 (1996).
16. Neonatal Des-TCRx2.4KerIV-K^b mice were injected intraperitoneally every second day with 20 μg of mouse E-selectin mAb UZ4 [rat immunoglobulin M (IgM)] and mouse P-selectin mAb RB40.34 (rat IgG1) from day 0 until day 7 and with 40 μg of antibody mixture from day 8 until day 15 [R. Hallmann, U. Zimmermann, L. M. Sorokin, L. Needham, K. Von der Mark, *Scand. J. Rheumatol. Suppl.* **101**, 107 (1995); R. Bosse and D. Vestweber, *Eur. J. Immunol.* **24**, 3019 (1994)]. Control groups were either noninjected or received an equivalent amount of purified irrelevant rat serum Ig containing IgM and IgG1. Four weeks after the treatment with mAbs, recipient mice were challenged with 10^5 P815.K^b tumor cells. The observed inhibition does not exclude the possibility that migration of naïve T cells into the neonatal skin is controlled by the expression of additional developmentally regulated adhesion molecules.
17. E- and P-selectin are up-regulated on vascular endothelium during inflammation. The presence of P-selectin in noninflamed tissue appears to be low and seems to require highly sensitive techniques for detection (*18*). In agreement with this finding, we observed low expression of P-selectin immunohistochemically in the skin of 5 of 10 newborn mice.
18. M. J. Eppihimer, B. Wolitzky, D. C. Anderson, M. A. Labow, D. N. Granger, *Circ. Res.* **79**, 560 (1996).
19. F. Powrie and D. Mason, *J. Exp. Med.* **172**, 1701 (1990); A. Miller, O. Lider, A. B. Roberts, M. B. Sporn, H. L. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 421 (1992); S. Qin *et al.*, *Science* **259**, 974 (1993); Y. Modigliani, A. Bandeira, A. Coutinho, *Immunol. Rev.* **149**, 155 (1996).
20. W. R. Heath, C. Kurts, J. F. A. P. Miller, F. R. Carbone, *J. Exp. Med.* **187**, 1549 (1998); C. Kurts *et al.*, *ibid.* **184**, 923 (1996).
21. We thank F. W. Alt for Rag-$2^{-/-}$ mice; J. Schöffel, A. Klevenz, S. Schmitt, and G. Küblbeck for excellent technical assistance; E. Armandola, T. Sacher, and A. Forde for critical reading of the manuscript; A. Frenznick, C. Ley, and S. Hartmann for animal husbandry; S. Copson for help with the manuscript; and G. Moldenhauer for providing control antibody. Supported by grants from Deutsche Forschungsgemeinschaft (SFB 405), the European Union (BIO4-CT-960077 and BIO4-97-2151), and VW-Stiftung (I/72422).

20 July 1998; accepted 14 October 1998