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Influence of the Major Histocompatability Complex on Positive Thymic Selection of $V_\beta17a^+$ T Cells

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A monoclonal antibody was used to show directly positive thymic selection of the T cell repertoire in mouse strains expressing the 17a β-chain variable domain ($V_\beta17a$) of the T cell receptor. In the absence of the potent tolerizing class II major histocompatability complex (MHC) molecule, I-E, peripheral expression of $V_\beta17a^+$ T cell receptors varied with the MHC haplotype of the mouse strain. In the most extreme case, H-2^q mice expressed high peripheral levels of $CD4^+$ $V_\beta17a^+$ T cells (14 to 19 percent), whereas H-2^b mice expressed low levels (3 to 4 percent). Analysis of (b × q)F_1 mice and chimeric mice showed that these differences were determined by positive thymic selection and implicated the thymic epithelium as the controlling cell type.

T CELL SPECIFICITY IS DIRECTED TOward antigen (Ag) that is complexed with a cell-surface molecule encoded by the MHC. Also, T cells preferentially recognize antigen in association with the organism's own MHC haplotype—a phenomenon termed self-MHC restriction—and yet cannot respond to self-MHC alone (self-tolerance). The T cell receptor (TCR) is a heterodimer, consisting of an α and a β chain. In addition to the TCR, which provides the specificity for Ag/MHC, T cells bear "accessory" molecules, CD4 and CD8, which bind monomorphic determinants on class II and class I MHC molecules, respectively. In the periphery, class I–restricted T cells are almost exclusively $CD8^+$, whereas class II–restricted cells are $CD4^+$. Thus, peripheral T cells are self-MHC–restricted, tolerant to self, and express an accessory molecule appropriate to the cell's MHC restriction. The process of T cell maturation in the thymus that produces such a precisely defined repertoire is believed to involve two selection events, a positive selection of T cells bearing TCRs biased toward self-MHC and a negative selection, or elimination, of T cells with autoreactive receptors.

Attempts to study thymic development have been hampered by the inability to measure populations of antigen-specific T cells directly. Recently, two new experimental approaches have been used: monoclonal antibodies (MAbs) that bind populations of TCRs that share some features of their structure and specificity (*1–3*) and TCR (αβ) transgenic mice that express predominantly one defined T cell specificity (*4–6*). We have examined the mechanism of tolerance to self-MHC with a MAb, KJ23a, that recognizes TCRs expressing the 17a β-chain variable region ($V_\beta17a$) (*1, 7, 8*). Because of the presence of a nonfunctional allele, $V_\beta17b$, in many strains of mice, $V_\beta17a$ has a restricted strain distribution (*9*). In $V_\beta17a^+$ strains of mice, TCRs that bear this V_β region react with high frequency to the class II MHC molecule, I-E. Antibody to $V_\beta17a$ could therefore be used to follow a population of T cells with a defined reactivity pattern. Mouse strains expressing I-E molecules had dramatically reduced peripheral expression of $V_\beta17a$ receptors. Examination of thymocytes in these I-E–tolerant mice showed that tolerance to self-MHC is mediated by clonal deletion of cells bearing autoreactive receptors during the immature, "double-positive" ($CD4^+/CD8^+$) stage of thymocyte maturation in the cortex (*1*). The presence of I-E on bone marrow–derived cells alone was sufficient to induce tolerance in the periphery (*7*).

Positive selection is less well characterized. The bias of the T cell repertoire to preferential recognition of antigen in the context of self-MHC is thought to reflect a positive selection event in the thymus, that is, only receptors capable of recognizing self-MHC (presumably in association with self-peptides) are given a signal to mature, but the evidence in support of positive selection has been indirect. Many (*10, 11*), but not all (*12*), studies in chimeric mice show that the peripheral repertoire is skewed toward recognition of antigen in the context of the MHC haplotype expressed in the thymus, and, in particular, the thymic epithelium [(*13*), but see (*14*)]. The interpretation of these studies has been made equivocal by the fact that the repertoire was measured by antigen reactivity (requiring immunization of experimentally manipulated animals), which might be influenced by peripheral and regulatory factors. Recently, positive selection was studied in TCR (αβ) transgenic mice (*5*). The receptor (as well as the accessory molecule) of the transgenic mice was preferentially expressed on T cells in mice of the MHC haplotype from which the receptor was originally cloned and not in two other haplotypes, implying positive selection by MHC in the absence of nominal antigen (*5*).

We report a direct demonstration of positive selection during thymocyte development, using the $V_\beta17a$ system previously used to define tolerance to self-MHC. An examination of peripheral $V_\beta17a$ TCR levels in mouse strains that do not express class II I-E molecules revealed a hierarchy of expression that correlated with MHC haplotype, such that H-2^q > H-2^s > H-2^b (Table 1A). Variation in expression was the greatest between H-2^q and H-2^b mice, particularly within the CD4 compartment of T cells (14 to 19% versus 3 to 4%). To confirm that the levels correlated with MHC haplotype and not strain-specific background genes, we bred the functional $V_\beta17a$ locus into B10 and B10.Q mice (*15*). The $V_\beta17a$ levels measured in these mice (Table 1A) confirmed the correlation with MHC haplotype.

The correlation between MHC and $V_\beta17a$ levels in the periphery is determined during thymic selection. We found that 10 to 11% of the immature cortical $\alpha\beta^+$ thymo-

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Table 1. Correlation of peripheral $V_\beta 17a$ levels with MHC haplotype in the absence of I-E tolerance. Cytofluorographic analysis was carried out on nylon wool–purified lymph node T cells by means of an Epics C flow cytometer (*1*). Percentages of $V_\beta 17a$-bearing T cells were defined with biotinylated KJ23a (*2*) and avidin-coupled phycoerythrin. CD4 and CD8 subpopulations were quantitated with directly fluorescein-conjugated antibodies GK1.5 (*25*) and 2.43 (*26*), respectively. As a control, peripheral levels of $V_\beta 3^+$ T cells were determined with MAb KJ25a (*16*). Total T cells are the sum of $CD4^+$ and $CD8^+$ T cells. The results shown represent analysis (average percentage ± SEM) of three or four animals assayed on different days. ND, not done.

Mouse strain	H-2 haplotype				Peripheral T cells expressing			
					$V_\beta 17a$ (%)			$V_\beta 3$ (%)
	K	A	E	D	Total T cells	$CD4^+$ T cells	$CD8^+$ T cells	Total T cells
A. SWR	q	q	–	q	16.0 ± 0.1	18.9 ± 1.0	5.0 ± 0.6	3.6 ± 0.1
B10.$Q_{\beta BR}$*	q	q	–	q	11.4 ± 0.3	14.4 ± 0.3	4.3 ± 0.4	4.5 ± 0.3
SJL	s	s	–	s	9.7 ± 0.2	10.7 ± 0.4	7.1 ± 0.1	ND
C57L	b	b	–	b	4.0 ± 0.2	2.6 ± 0.1	6.4 ± 0.2	6.0 ± 0.4
B10.βJ†	b	b	–	b	5.0 ± 0.2	3.6 ± 0.3	8.4 ± 0.4	4.8 ± 0.7
B. (C57L × SWR)F_1	b/q	b/q	–	b/q	8.8 ± 0.8	11.2 ± 0.5	5.2 ± 0.3	4.8 ± 0.2
(B10.βJ × B10.$Q_{\beta BR}$)F_1	b/q	b/q	–	b/q	9.7 ± 0.3	13.1 ± 0.3	5.2 ± 0.3	4.5 ± 0.2

*The T cell receptor β locus from C57BR (βBR) was crossed onto B10.Q mice to produce F_2 mice homozygous for H-2^q and the βBR locus. Although these mice are neither congenic nor inbred, there was a 100% correlation between MHC haplotype and levels of $V_\beta 17a$ expression (*15*). †B10 mice were made congenic for the T cell receptor β locus from SJL mice (βJ). The mice analyzed here were from the sixth generation backcross.

Table 2. Variation in peripheral levels of $V_\beta 17a^+$ T cells in H-2^q and H-2^b mice is determined in the thymus. Thymocytes from B10.Q βBR and B10.βJ mice were stained for $V_\beta 17$ with biotinylated KJ23a and total αβ receptors with biotinylated 597, a MAb that reacts with all $\alpha\beta^+$ TCR (*27*). The $V_\beta 17a$ levels on thymocytes were calculated as a percentage of the total αβ receptors. Cortical and medullary thymocyte populations were distinguished on the basis of their staining intensity (*28*). In order to quantitate more readily the two populations, thymocytes were cultured in tissue culture media at 37°C for 1 to 4 hours, a procedure that increases the density of receptor on the immature cells, making them more easily distinguished from autofluorescence and nonstained cells, but still easily distinguishable from the higher density medullary population (*29*). Results are shown as average percentage; three mice in each group were assayed.

Strain	Cortical thymocytes			Medullary thymocytes		
	$V_\beta 17a$ (%)	αβ (%)	$V_\beta 17a$ (% of αβ)	$V_\beta 17a$ (%)	αβ (%)	$V_\beta 17a$ (% of αβ)
B10.$Q_{\beta BR}$	4.6	43.2	10.5	1.19	10.4	11.5
	3.9	39.4	9.8	1.03	7.5	13.8
	4.1	43.7	9.4	0.93	7.0	13.2
Mean ± SEM			9.9 ± 0.3			12.8 ± 0.7
B10.βJ	4.6	41.4	11.1	0.40	7.7	5.1
	5.0	40.6	12.4	0.33	7.0	4.7
	4.7	43.6	10.8	0.38	7.2	5.2
Mean ± SEM			11.4 ± 0.5			5.0 ± 0.2

cytes in both B10.βJ and B10.$Q_{\beta BR}$ strains of mice express $V_\beta 17a$, whereas the percentages of $V_\beta 17a^+$ mature medullary thymocytes differ between the two strains and are similar to those found on peripheral T cells in the two types of mice (Table 2). In this analysis, we were unable to determine whether positive selection or tolerance caused this difference. However, evidence in favor of control by positive selection was obtained by studying (b × q)F_1 mice (Table 1B). If the low level of $CD4^+$ $V_\beta 17a^+$ expression in H-2^b mice were due to tolerance, we would predict that expression in the (b × q)F_1 mouse would be suppressed, as was previously shown for tolerance to I-E in this system (*1*). In contrast, an intermediate to high peripheral level of $CD4^+$ $V\beta 17a^+$ expression in the (b × q)F_1 mice was found (Table 1B). Thus, the difference in levels of $CD4^+$ $V_\beta 17a^+$ TCR in H-2^q and H-2^b mice may be controlled by positive selection in the thymus, presumably by I-A^q versus I-A^b.

To examine this further, we constructed $F_1 \rightarrow$ parent chimeric mice. In this system, (b × q)F_1 (β10.βJ × B10.$Q_{\beta BR}$) bone marrow cells were injected into irradiated B10 or B10.Q recipient mice and selected in the H-2^b or H-2^q host. As the B10 and B10.Q recipients lack a functional $V_\beta 17$ gene, they do not express $V_\beta 17a^+$ T cells. Thus, all of the $V_\beta 17a^+$ T cells detected in the chimeric mice were generated from the donor bone marrow cells. The peripheral level of $V_\beta 17a^+$ T cells, expressed as a percentage of the donor cells in the chimera, reflected the MHC type of the irradiated host, thus supporting a role for positive selection in determining $V_\beta 17a^+$ TCR levels in these mice (Table 3). A second antibody, KJ25, was used to quantitate $V_\beta 3^+$ cells in the chimeric mice (*16*). Comparable levels of $V_\beta 3^+$ T cells were found in both sets of chimeric mice, suggesting that the variation in $V_\beta 17a$ levels was due to a specific selection event.

The role of thymic epithelium in mediating positive selection has strong (*13*), although not universal (*14*), support. We examined this question by measuring peripheral levels of $V_\beta 17a^+$ T cells in allogeneic and syngeneic thymic chimeras. Recipient mice (H-2^b or H-2^q) were thymectomized, depleted of T cells, irradiated, reconstituted with syngeneic T cell–depleted bone marrow cells, and subsequently grafted with allogeneic or syngeneic 14-day embryonic thymus lobes that had been cultured in the presence of deoxyguanosine for 5 days to eliminate bone marrow–derived cells (*17*). Again, the chimeras were constructed such that only the reconstituting bone marrow cells had the functional $V_\beta 17a$ locus, ensuring that all the $V_\beta 17a^+$ cells repopulating the periphery matured under the influence of the grafted thymus. In addition, host cells and MHC syngeneic donor cells were easily distinguished by a non–H-2 polymorphism, Lpg 100 (*18*), for the H-2^q mice and Lyt1 (*19*) for the H-2^b mice. Thus, B10.Q mice (H-2^q, Lpg 100^-, $V_\beta 17b$) were reconstituted with bone marrow cells from SWR mice (H-2^q, Lpg 100^+, $V_\beta 17a$) and B6Lyt1.1 mice (H-2^b, Lyt1.1, $V_\beta 17b$) were reconstituted with bone marrow cells from C57L mice (H-2^b, Lyt1.2, $V_\beta 17a$). The $V_\beta 17a^+$ T cells in the periphery of the chimeras were quantitated 6 weeks after the thymus transplant, and expressed as a percentage of the donor T cells. The results support the role of thymic epithelial cells in mediating positive selection (Table 4). The chimeras given syngeneic thymus grafts expressed the predicted peripheral $CD4^+$ $V_\beta 17a^+$ T cell levels similar to those of unmanipulated H-2^q and H-2^b animals. The presence of an H-2^q thymus graft in an otherwise H-2^b animal resulted in peripheral $CD4^+$ $V_\beta 17a$ levels skewed toward the H-2^q level, whereas an H-2^b thymus graft in an H-2^q animal significantly lowered the level of $CD4^+V_\beta 17a^+$ T cells

from that expressed in an H-2^q animal. This skewing of the peripheral Vβ17a levels in the allogeneic chimeras toward the level characteristic of the H-2 haplotype expressed in the thymus graft (presumably by thymic epithelial cells) confirms the ability of the thymic epithelium to mediate positive selection.

The allogeneic chimeras, however, did not fully attain the levels of V_β17a in the periphery of the control syngeneic thymic chimeras nor in the $F_1 \rightarrow$ parent bone marrow chimeras. It is possible that bone marrow–derived cells may also function in positive selection, and the allogeneic chimeras may reflect the intermediate level of positive selection mediated by the different haplotypes expressed on the bone marrow cells and thymic epithelial cells, resulting in an F_1 level of selection. Alternatively, the peripheral V_β17 level in the allogeneic chimeras may reflect separate mechanisms of positive and negative selection, mediated independently by the two MHC haplotypes. For example, the failure of the H-2^b mouse grafted with an H-2^q thymus to attain the V_β17 level of an H-2^q mouse grafted with an H-2^q thymus may reflect subsequent tolerance of H-2^q–selected cells on H-2^b–expressing bone marrow–derived cells. This is an unlikely explanation because the (b × q)$F_1 \rightarrow$ q chimeras, which would also permit H-2^b tolerance on an H-2^q–selected population, did express the full level of V_β17 cells predicted by selection on H-2^q. Another possibility relates to the biological limitation of allogeneic chimeras, originally noted by Zinkernagel (*20*). Complete T cell maturation may involve an extra-thymic stage that is dependent on MHC antigens shared between the thymus and lymphohemopoietic cells (*21, 22*). Because of this preference for syngeneic maturation in the periphery, in an allogeneic chimera, even a small number of host precursor cells that escaped irradiation or a small number of T cells transferred with the graft will preferentially expand, effectively lowering the percent of thymic (allo)-educated cells in the periphery.

Another unresolved issue in positive thymic selection concerns the determination of the CD4 or CD8 property of the developing T cell. Treatment with MAb to block CD4 molecules in the thymus prevented the development of CD4$^+$ peripheral T cells (*23*), suggesting a role for CD4 in positive selection. Studies with transgenic mice have shown that the CD4/CD8 identity of a cell is determined during thymic selection by interaction of the TCR and MHC (*5, 6*). In our studies, identifying only the TCR β chain with a MAb, we found that positive selection of V_β17a$^+$ TCR in H-2^q mice strongly favored CD4$^+$ cells. Whereas in B10.Q mice, the overall CD4:CD8 ratio was 70:30, within the V_β17a$^+$ population of T cells the ratio was skewed to 90:10. Also, although F_1 mice expressed a ratio of 75:25 within the V_β17a$^+$ population, the characteristic 90:10 ratio was reestablished during selection on H-2^q in $F_1 \rightarrow$ H-2^q chimeric mice. This indicated that V_β17a$^+$ cells that matured in an H-2^q thymus tended to be CD4$^+$, establishing a link between positive selection and accessory molecules. The results in the $F_1 \rightarrow$ b chimeras were not as marked, which may merely reflect the relatively poor selection of V_β17a$^+$ TCR by H-2^b. Thus, the accumulating data imply that CD4/CD8 determination is an integral part of the selection process that involves interaction among the TCR, accessory molecules, and MHC molecules.

Table 3. Peripheral V_β17a levels in $F_1 \rightarrow$ parent chimeras are determined by the MHC haplotype of the parent. Bone marrow chimeric mice were established as previously described (*11*). T cell–depleted recipient B10 or B10.Q mice received 750 R of irradiation and were reconstituted with (B10.βJ × B10.Q$_{\beta BR}$)F_1, T cell–depleted bone marrow cells. Nylon wool–purified peripheral lymph node cells were analyzed 6 weeks after reconstitution. The percentage chimerism within the total T cells and within the CD4 and CD8 compartments was determined with two-color staining with the use of fluorescein-conjugated MAbs to H-2^b (*30*) and H-2^q (*31*), and biotinylated antibodies to CD4 and CD8 (GK1.5 and 53.6 or 2.43). Donor cells varied between 65 and 98% of all T cells. Peripheral V_β17a levels were determined with MAb KJ23a as described in Table 1 and calculated as a percentage of donor T cells, because only the donor strains carry the functional gene for V_β17. Peripheral V_β3 levels, as a percentage of total T cells, were determined as described in Table 1. Values are means ± SEM of six individual mice, assayed on four separate days.

$F_1 \rightarrow$ parent chimeric mice	*n*	Peripheral T cells expressing			
		V_β17a (% donor cells)			V_β3 (% total cells)
		Total T cells	CD4$^+$ T cells	CD8$^+$ T cells	Total T cells
(B10.βJ × B10.Q$_{\beta BR}$)$F_1 \rightarrow$ B10	6	4.1 ± 0.1	3.4 ± 0.2	6.7 ± 0.5	4.8 ± 0.4
(B10.βJ × B10.Q$_{\beta BR}$)$F_1 \rightarrow$ B10.Q	6	17.4 ± 0.7	20.2 ± 0.6	10.0 ± 1.8	4.2 ± 0.2

Table 4. Peripheral V_β17a levels in thymus chimeras reflect the MHC haplotype of thymic epithelial cells. Thymus chimeras were made as previously described (*7*). Recipient mice that have a defect in the structural gene for V_β17 (V_β17b) were thymectomized at 4 weeks of age. They were subsequently T cell depleted, given 750 R of irradiation, and reconstituted with syngeneic bone marrow from mouse strains that have a functional V_β17 gene (V_β17a). The mouse stains used were chosen to allow donor and host cells in the chimeras to be distinguished. Thus, B10.Q mice (H-2^q, V_β17b, Lpg 100$^-$) were reconstituted with SWR bone marrow (H-2^q, V_β17a, Lpg 100$^+$) and B6Lyt1.1 mice (H-2^b, V_β17b, Lyt1.1) were reconstituted with C57L bone marrow (H-2^b, V_β17a, Lyt1.2). One week after bone marrow reconstitution, the mice were grafted with six thymic lobes from day 14 embryos from either C57BL/6 or B10.Q mice that had been cultured in 1.3 m*M* deoxyguanosine for 5 days before implantation in order to eliminate lymphoid elements (*17*). Six weeks after the thymic transplants, the mice were analyzed by flow cytometry. Chimerism was determined by means of two-color staining with biotinylated 30-C7, to detect Lpg 100 (*18*) and fluorescein-conjugated antibodies to CD4 (GK1.5) and to CD8 (53.6) for the H-2^q chimeras, and antibodies to Lyt1.1 and to 1.2 in conjunction with a fluorescein-conjugated antibody to murine immunoglobulin G_{2b} (*19*) and biotinylated antibodies to CD8 and to CD4 for the H-2^b chimeras. The percent chimerism ranged between 60 and 98%. V_β17a and V_β3 levels were determined with KJ23a and KJ25a, as in Tables 1 and 3.

AT×BM* recipient mouse	Bone marrow source	Thymus graft	*n*	Peripheral T cells expressing			
				V_β17a (% donor cells)			V_β3 (% total cells)
				Total T cells	CD4$^+$ T cells	CD8$^+$ T cells	Total T cells
B6Lyt1.1 (H-2^b)†	C57L (H-2^b)	C57Bl/6 (H-2^b)	8	3.9 ± 0.3‡	3.5 ± 0.4	4.9 ± 0.4	7.0 ± 0.4
B6Lyt1.1 (H-2^b)	C57L (H-2^b)	B10.Q (H-2^q)	6	11.4 ± 0.8	13.6 ± 0.8	5.8 ± 1.0	6.9 ± 1.2
B10.Q (H-2^q)	SWR (H-2^q)	B10.Q (H-2^q)	6	16.4 ± 1.9	20.7 ± 0.4	5.5 ± 1.3	4.7 ± 0.6
B10.Q (H-2^q)	SWR (H-2^q)	C57Bl/6 (H-2^b)	5	7.9 ± 0.6	7.7 ± 1.1	10.2 ± 0.7	5.7 ± 0.6

*AT×BM, adult thymectomized, irradiated, bone marrow reconstituted. †H-2 haplotype stated in parentheses. ‡Average percentage ± SEM from five to eight individual mice, assayed on four separate days.

Since the phenomenon of self-MHC restriction was first described, positive selection had been inferred to be the mechanism, but the evidence was always indirect. Now positive selection has been formally demonstrated. The mechanism involves the interaction of TCR chains, accessory molecules, and MHC during thymus development.

Note added in proof: Recently, positive selection has been proposed to explain the correlation between levels of T cells bearing $V_\beta 6^+$ TCR and MHC class II I-E molecules in MHC congenic and F_1 mice (*24*).

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Chromosome 17 Deletions and p53 Gene Mutations in Colorectal Carcinomas

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Previous studies have demonstrated that allelic deletions of the short arm of chromosome 17 occur in over 75% of colorectal carcinomas. Twenty chromosome 17p markers were used to localize the common region of deletion in these tumors to a region contained within bands 17p12 to 17p13.3. This region contains the gene for the transformation-associated protein p53. Southern and Northern blot hybridization experiments provided no evidence for gross alterations of the p53 gene or surrounding sequences. As a more rigorous test of the possibility that p53 was a target of the deletions, the p53 coding regions from two tumors were analyzed; these two tumors, like most colorectal carcinomas, had allelic deletions of chromosome 17p and expressed considerable amounts of p53 messenger RNA from the remaining allele. The remaining p53 allele was mutated in both tumors, with an alanine substituted for valine at codon 143 of one tumor and a histidine substituted for arginine at codon 175 of the second tumor. Both mutations occurred in a highly conserved region of the p53 gene that was previously found to be mutated in murine p53 oncogenes. The data suggest that p53 gene mutations may be involved in colorectal neoplasia, perhaps through inactivation of a tumor suppressor function of the wild-type p53 gene.

RECENT STUDIES HAVE ELUCIDATED several genetic alterations that occur during the development of colorectal tumors (*1–3*), the most common of which are deletions of the short arm of chromosome 17. While some genetic alterations, such as *RAS* mutations, appear to occur relatively early during colorectal tumor development, chromosome 17p deletions are often late events associated with the transition from the benign (adenomatous) to the malignant (carcinomatous) state (*1*). Because carcinomas are often lethal, while the precursor adenomas are uniformly curable, the delineation of the molecular events mediating this transition are of considerable importance. The occurrence of allelic deletions of chromosome 17p in a wide variety of cancers besides those of the colon, including those of the breast and lung, further emphasizes the importance of identifying genes on 17p that are involved in the neoplastic process (*4*).

Our approach to this identification was based on first defining a small region of chromosome 17p that is commonly lost in different colorectal carcinomas. Twenty DNA probes detecting restriction fragment length polymorphisms (RFLPs) on chromosome 17p were used to examine the patterns of allelic losses in colorectal tumors. These probes have been mapped to seven discrete regions of 17p on the basis of their hybridization to human-rodent somatic cell hybrids containing parts of chromosome 17p (*5*). DNA was obtained from 58 carcinoma specimens and compared to DNA from adjacent normal colonic mucosa. Allelic losses were scored if either of the two alleles present in the normal cells was absent in the DNA from the tumor cells. Allelic deletions can be difficult to detect in DNA prepared from whole tumors because most solid tumors contain a significant number of non-neoplastic stromal and inflammatory cells. For this reason, regions of tumors containing a high proportion of neoplastic cells were isolated, and DNA was prepared from cryostat sections of these regions as described (*6*).

The two parental alleles could be distinguished in the normal mucosa of each patient by at least 5 of the 20 RFLP markers (the "informative" markers for each case). Seventy-seven percent of the tumors exhibited allelic losses of at least three markers. Studies of eight tumors that retained heterozygosity for some but not all markers on

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