genes introduced by transformation (*12, 13*) into yeast may be expressed and channel activity recorded. The effect of site-specific mutagenesis of the channel gene (*14*) on channel function could then be analyzed.

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Altered K^+ Channel Expression in Abnormal T Lymphocytes from Mice with the *lpr* Gene Mutation

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The observation that voltage-dependent K^+ channels are required for activation of human T lymphocytes suggests that pathological conditions involving abnormal mitogen responses might be reflected in ion channel abnormalities. Gigaohm seal techniques were used to study T cells from MRL/MpJ–*lpr/lpr* mice; these mice develop generalized lymphoproliferation of functionally and phenotypically abnormal T cells and a disease resembling human systemic lupus erythematosus. The number and predominant type of K^+ channels in T cells from these mice differ dramatically from those in T cells from control strains and a congenic strain lacking the *lpr* gene locus. Thus an abnormal pattern of ion channel expression has now been associated with a genetic defect in cells of the immune system.

RECENT STUDIES WITH THE GIgaohm seal technique indicate that the most abundant ion channel in human and murine T lymphocytes is a voltage-gated K^+ channel closely resembling the delayed rectifier of muscle and nerve (*1–5*). Sodium channels have been detected only infrequently in T lymphocytes, and calcium channels have not been observed thus far. Several types of evidence suggest that K^+ channels play a necessary role during human T-lymphocyte activation and proliferation (*1, 4, 6*). In this report we show that K^+ channels in murine T cells play a similar role. In addition, we have taken advantage of the existence of a strain of mice, MRL/MpJ–*lpr/lpr* (MRL-l), with genetically induced abnormalities of T lymphocytes (*7*), to further explore the involvement of K^+ channels in T-cell proliferation.

MRL-l mice that are homozygous for the autosomal recessive *lpr* mutation, develop severe lymph node enlargement early in life that reflects polyclonal expansion of a T-cell subset with a unique set of cell surface markers (*8*). The abnormal subset of T cells does not actively cycle in vivo, and may represent cells arrested at an immature stage of differentiation (*9*). These cells display impaired interleukin-2 production and proliferation in response to mitogens in vitro (*7, 10*). The congenic control strain, MRL/MpJ–+/+ (MRL-n), which differs from MRL-l mice only in lacking the *lpr* mutation, does not develop lymphoproliferation, and T lymphocytes from these mice express a normal phenotype and respond normally to mitogens in vitro (*7–10*). In addition, they exhibit a lupus-like disease much later in life than MRL-l mice (*7*). Thus, young congenic MRL-n mice represent a useful control for comparative studies.

Figure 1 shows outward K^+ currents recorded from individual MRL-l and MRL-n T lymphocytes. Superficially, these K^+ currents resemble those in human T lymphocytes (*3*). MRL-n T cells typically had on the order of ten K^+ channels per cell, with a low average (±SEM) maximum K^+ conductance, g_K, of 0.37 ± 0.10 nS ($n = 38$). The g_K of 26 T cells from four other control mouse strains (CBA/J, C57BL/6J, BALB/c, and C3H/HeJ) that do not spontaneously develop lymphadenopathy or autoimmune manifestations ranged from 0.01 to 0.54 nS (*5*). The g_K was similar in T lymphocytes from lymph node and spleen among the five control strains; therefore these results have been pooled. K^+ currents in T cells from MRL-l mice were substantially larger than in the control strains. The average ± SEM g_K for all the MRL-l T lymphocytes was 4.6 ± 0.6 nS ($n = 31$). T cells from MRL-l lymph nodes ($n = 23$) had uniformly high g_K values. The g_K in MRL-l splenic T cells was more variable, with two of eight cells having low g_K values in the range for T cells from normal mice, probably reflecting normal T cells in the population. The spleens from MRL-l mice have a lower fraction of cells with the aberrant phenotype (*8, 11*). In one experiment, the fraction of phenotypically abnormal splenic T cells was enriched by selecting for Lyt-1^+2^- T cells (*11*). The three purified MRL-l splenic T cells studied had a consistently high g_K, comparable to that in lymph node MRL-l T cells. In contrast, two purified Lyt-1^+2^- T cells from control MRL-n mice had very low g_K values, in the range for normal mouse T cells. Dividing the g_K by the measured single K^+ channel conductance gave an estimate of 220 K^+ channels in a typical MRL-l T lymphocyte and about 12 K^+ channels per MRL-n T lymphocyte. Thus, T cells from diseased MRL-l mice have more than an order of magnitude more K^+ channels than normal mouse T lymphocytes. With the exception of Na^+ channels sometimes ob-

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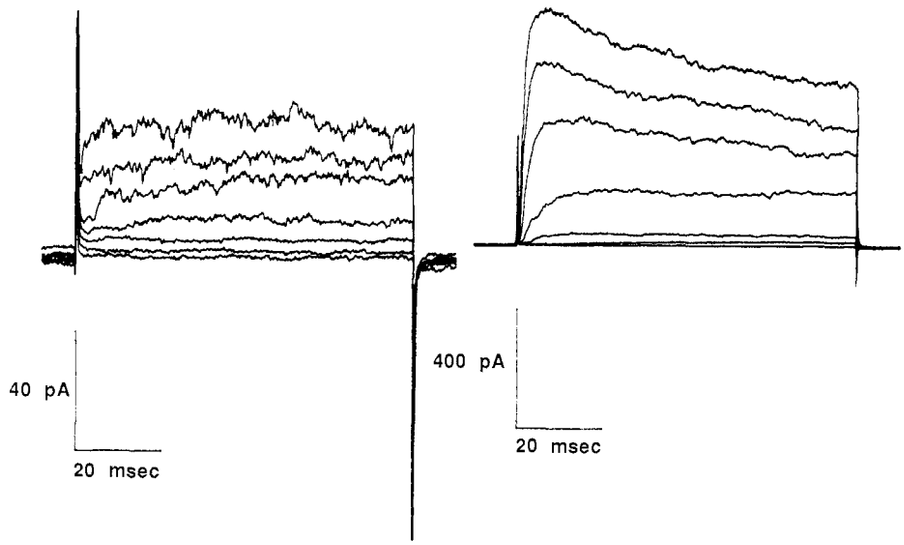

Fig. 1. Potassium currents recorded in the whole-cell configuration in T lymphocytes from MRL-n (left) and MRL-l (right) mice. The membrane potential was held at −80 mV and depolarizing pulses were applied every 20 seconds in 20-mV steps up to +60 mV. T cells were isolated from single-cell suspensions prepared from spleen or lymph nodes, by passage through a nylon wool column. MRL-n mice studied were from about 1.5 to 7 months of age. Experiments were performed at room temperature (20–23°C) with the gigaohm seal voltage clamp technique (*1, 3*). The composition of the bathing solution (Ringer solution) was: 160 m*M* NaCl, 4.5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 5 m*M* Hepes, *p*H 7.4. The pipette solution was mainly KF, with a free calcium concentration of less than 2 n*M* (*1, 3*). MRL-l T cells had larger mean diameter (6.3 μm) and input capacity (1.28 pF) than MRL-n T cells (5.7 μm and 0.92 pF, respectively). The K^+ channel density is about 2 per square micrometer (200 per picofarad) in MRL-l and 0.1 per square micrometer (12 per picofarad) in MRL-n T cells.

served in MRL-n T cells (*5*), no other types of ion channels were consistently observed in any strain.

Single-channel recording from isolated membrane patches or from whole cells revealed two distinct types of K^+ channels, called *l* (large or *lpr*) and *n* (normal). Figure 2 illustrates some of the properties of these two types of K^+ channels in an MRL-n T cell. The unitary conductance of type *l* channels was about 21 pS and the conductance of the type *n* channels was about 12 pS. Type *l* channels characteristically opened at more positive potentials than type *n* channels. The two types of K^+ channels also have very different sensitivities to blockage by tetraethylammonium (TEA). Half of the conductance of type *n* channels was blocked by about 8 to 16 m*M* TEA, which is similar to the sensitivity of K^+ channels in human T lymphocytes (*1*). Type *l* channels were about 100 times more sensitive, with half-block at 50 to 100 μ*M*. These and other differences in the properties of type *l* and type *n* K^+ channels are described in detail elsewhere (*12*). The pharmacological difference, in conjunction with other macroscopic properties, made it possible to determine which type of channel predominated in a given cell. Both type *n* and *l* K^+ channels were present in small numbers in T lymphocytes from control mice. Most cells had predominantly one or the other type of K^+ channel, although both can be present in the same cell (Fig. 2). Of T lymphocytes from control MRL-n mice, roughly half expressed mainly type *l* and half mainly type *n* K^+ channels. It remains to be determined whether type *l* and *n* channels correlate with specific markers in normal mouse T cells. T-cell activation selectively enhances the number of type *n* channels by more than an order of magnitude (*4, 5*). In contrast, macroscopic and unitary K^+ currents in diseased MRL-l mice were almost always type *l*. Thus, T lymphocytes from diseased MRL-l mice differed from normal resting or activated T cells in having an abundance of type *l* K^+ channels.

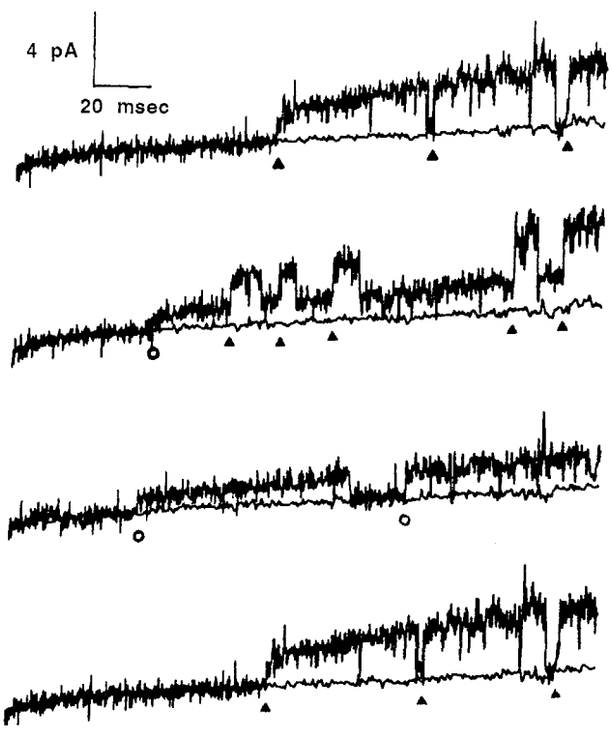

Fig. 2. Single K^+ channel events recorded during voltage-clamp "ramps" in the whole-cell configuration in an MRL-n T lymphocyte. The cell was held at −50 mV to inactivate most of the K^+ channels present, and the potential was "ramped" repeatedly from −50 mV to +80 mV. The four records illustrate currents recorded during single ramps, with the averaged current during records in which no channels opened superimposed as a lighter line. The triangles indicate openings of the larger type *l* K^+ channels, the circles mark openings of smaller type *n* K^+ channels. Both channel types frequently "flicker" closed and rapidly reopen; no attempt was made to mark each such event.

The altered expression of K^+ channels in MRL-l T cells parallels the disease process. In MRL-l mice 6 weeks of age, prior to the onset of clinically identifiable lymphadenopathy (*7*), only 5 to 6% of lymph node cells are phenotypically abnormal (*8*), and the T cells respond normally to mitogens (*10*). In T cells from MRL-l mice of this age, K^+ channel expression was similar to that in the congenic strain MRL-n. Most T cells (8 of 12) from young MRL-l mice had small g_K, 0.4 nS or less, and (like MRL-n) about half of the cells expressed mainly type *l* and half type *n* K^+ channels. After the onset of disease in mice >4 months of age, when 80 to 90% of the lymph node cells are phenotypically and functionally abnormal (*8, 10, 11*), most of the 31 MRL-l T cells studied (82%) had large g_K (>1 nS), and all but one of the cells categorized expressed type *l* K^+ channels. Thus, the fraction of MRL-l T lymphocytes expressing large numbers of type *l* K^+ channels increases with age in parallel with the expansion of the phenotypically and functionally abnormal T-lymphocyte subset, and apparently is one characteristic of these cells.

MRL-l T lymphocytes respond poorly to the T-cell mitogen, Concanavalin A (Con

A), compared with MRL-n T lymphocytes (Fig. 3A). Con A–stimulated incorporation of [^{3}H]thymidine in MRL-n T cells was inhibited in a dose-dependent manner by verapamil, diltiazem, quinine, 4-aminopyridine, and TEA, at concentrations that block K^+ channels in these cells (Fig. 3B). Similar results have been observed with human T lymphocytes activated with the mitogen phytohemagglutinin, allogeneic cells, or with the mitogenic antibody OKT3 (*1, 4, 6*). Con A–stimulated incorporation of [^{3}H]thymidine was inhibited by TEA only at concentrations that block type *n* K^+ channels, but not at lower concentrations that completely block type *l* K^+ channels, suggesting that type *n* but not type *l* K^+ channels are required for T-cell activation. It is unlikely that TEA exerts its inhibitory activation via an indirect effect on contaminating non–T cells in the preparation, since TEA inhibited mitogen-stimulated proliferation of a pure T-cell clonal population (D10.G4) that expresses type *n* K^+ channels with the same potency as for blockage of MRL-n T-cell mitogenesis (*13*). TEA (25 m*M*) also suppressed interleukin-2 production by about 68% in two experiments (measured with the CTLL-2 cell line) (*14*), but did not significantly block Con A–stimulated interleukin-2 receptor expression (determined with the 7D4 monoclonal antibody). A similar pattern of results has been obtained in human T lymphocytes (*1, 6*). Thus, these data, along with the observation that murine T-cell activation is accompanied by a selective induction of type *n* K^+ channels (*4, 5*), suggest that type *n* K^+ channels are required for mitogenesis in control T lymphocytes.

The relationship between the abnormal type *l* K^+ channel expression and the deficient mitogen responsiveness of MRL-l T cells in vitro remains unclear. One possibility is that the presence of type *l* channels is somehow inhibitory to mitogenesis. However, an intermediate dose of TEA (1 m*M*), which blocks virtually all type *l* K^+ channels with negligible effects on type *n* channels, did not restore Con A–stimulated [^{3}H]thymidine incorporation into MRL-l T lymphocytes to the level in MRL-n T lymphocytes (MRL-l cells: no TEA, 894 ± 120 count/min; TEA, 495 ± 117 count/min; MRL-n cells: 10,169 ± 1384 count/min; mean ± SEM of three experiments, each done in triplicate on cells from an individual mouse). These results indicate that type *l* K^+ conductance does not directly inhibit activation in MRL-l mice. Second, it might be imagined that type *l* channels do not respond to mitogen, as type *n* channels do (*1, 3, 4*), with enhanced opening of the channel after adding the mitogen. Con A rapidly shifted the voltage dependence of type *l* K^+ channels to about 10-mV more negative potentials, a mitogen response similar to that in human T cells. Even after a Con A–induced hyperpolarizing shift, type *l* K^+ channels still open at more positive potentials than type *n* K^+ channels. Thus it remains possible that differences in channel voltage dependence or membrane potential may contribute to the impairment of mitogen activation of MRL-l T cells in vitro, since membrane depolarization has been reported to inhibit T-cell activation (*15*).

Here we report that a mutation at the single gene locus, *lpr*, is associated with altered expression of voltage-gated K^+ channels in mitogen-unresponsive T lymphocytes from MRL-l mice. Numerous type *l* K^+ channels are present in functionally and phenotypically abnormal T cells from MRL-l mice, suggesting that augmented expression of type *l* K^+ channels is another characteristic of the aberrant expanded T-cell subset. This subset may be present in minute numbers in normal mice, perhaps representing cells at an immature stage of differentiation (*9*). Alternatively, the *lpr* mutation may modify a gene that regulates the number and characteristics of K^+ channels in T lymphocytes. Single gene mutations resulting in alterations in K^+ channels associated with behavioral abnormalities have been described in *Drosophila* (*16*). Understanding the relationship between the *lpr* gene locus and the expression of large numbers of type *l* K^+ channels may provide insight into the pathogenesis of lymphoproliferation, as well as mechanisms controlling ion channel expression during differentiation.

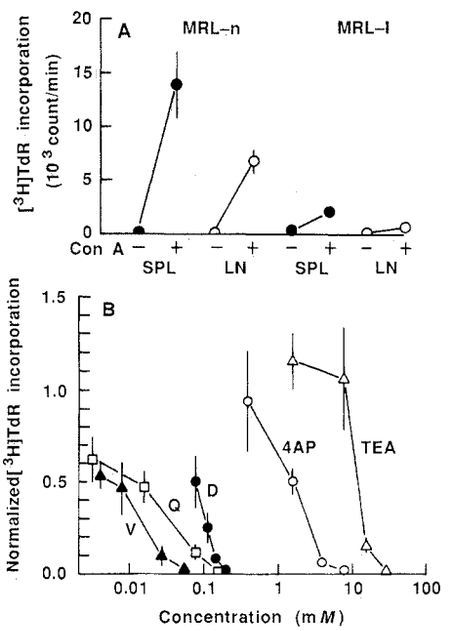

Fig. 3. (A) Incorporation of [^{3}H]thymidine (*6*) in the absence (−) or presence (+) of Con A (2 μg/ml), in splenic (SPL) or lymph node (LN) T cells from MRL-n and MRL-l mice. Mean ± SEM of four experiments, each done in triplicate on cells from different mice. (B) Inhibition of Con A–stimulated [^{3}H]thymidine incorporation by verapamil (V), quinine (Q), diltiazem (D), 4-aminopyridine (4AP), and TEA, in MRL-n lymph node T cells. Results with splenic T cells were similar. The 4AP and quinine were purchased from Sigma, St. Louis, MO; TEA was purchased from Eastman Kodak and was recrystallized prior to use. A 50% reduction of peak K^+ currents occurred in the range of 4 to 40 μ*M* for verapamil, quinine, and diltiazem and at ≤200 μ*M* for 4AP. Inhibition of mitogenesis occurred at concentrations that block type *n* K^+ channels. Each data point represents the mean ± SEM of [^{3}H]thymidine incorporation (normalized to that in the absence of blockers) in four experiments; each experiment was done in triplicate on cells from an individual mouse.

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High Nitrite Levels off Northern Peru: A Signal of Instability in the Marine Denitrification Rate

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During February and March 1985, nitrite levels along the northern (approximately 7° to 10°S) Peruvian coast were unusually high. These accumulations occurred in oxygen-deficient waters, suggesting intensified denitrification. In a shallow offshore nitrite maximum, concentrations were as high as 23 micromoles per liter (a record high). Causes for the unusual conditions may include a cold anomaly that followed the 1982–83 El Niño. The removal of combined nitrogen (approximately 3 to 10 trillion grams of nitrogen per year) within zones of new or enhanced denitrification observed between 7° to 16°S suggests a significant increase in oceanic denitrification.

THEORETICAL CONSIDERATIONS SUGGEST that the loss of combined nitrogen from the sea by denitrification may be highly variable (*1*). Observations off Peru and Chile support this assertion. Investigators have consistently encountered oxygen-deficient (O_2 less than ~0.05 ml of oxygen per liter) waters with embedded nitrite maxima, and have shown that denitrification is a major process in these zones (*2–5*). These features are, however, highly variable. For example, after the 1972 El Niño and the collapse of the anchoveta stocks, denitrification may have increased by $\sim 10 \times 10^{12}$ g of nitrogen per year (*2*) to a rate of $\sim 25 \times 10^{12}$ g/year. This value is 20 to 60% of the total marine denitrification rate (*4, 6*) and ~20% of the largest estimate for the removal rate of fixed nitrogen from the ocean by all processes (*6*).

From 1 February to 5 March 1985, observations (*7*) off Peru (Fig. 1) suggested another increase in denitrification. Between 7° and 10°S, nitrite levels in oxygen-deficient waters (Figs. 2 and 3) were unusually high (*8*). High nitrite concentrations occurred (i) in a maximum centered at depths of 200 to 300 m, (ii) in a shallow offshore maximum where extreme values were observed (maximum value of 23 μM at 24 m at station 16), and (iii) over the shelf. High concentrations have occurred over the shelf (*9*), but a maximum at 200 to 300 m is rare at this latitude, and the nitrite concentrations in the shallow offshore maximum appear to be the highest ever observed in the open ocean. Maximum nitrous oxide concentrations (Fig. 2) were also high, and the wide range of values (0 to 8.6 μg/liter) implied rapid turnover (*6*).

Observations from northern Peru similar to ours were taken from February to March 1974 and February to May 1975 (*10, 11*) during cold anomalies as indicated by temperatures measured at 7°43′S (*12*). During 1974, a nitrite concentration of ~11 μM was observed offshore at a depth of 50 m, and a zone of complete nitrate removal and sulfide accumulation occurred over the shelf. During 1975, suggestions of the initial stages of shallow offshore nitrite maxima and maxima below 100 m were encountered.

During 1985, nitrite concentrations greater than 1 μM were found everywhere in the oxygen-deficient waters of our study region (Fig. 2) except near 11°S where an onshore flow of low-nitrite waters separated the nitrite maxima. The onshore flow occurred near the typical northern boundary of the main secondary nitrite maximum normally found between ~100 and 400 m and between 10° and 25°S (*2*), suggesting that the deep maximum found in the north is not an expansion of this feature. We have omitted the section taken at 15°S (Fig. 1), but intense denitrification was indicated by high nitrate deficits (*13*) at shallow depths. In addition, the only site of complete denitrification (total consumption of nitrate and nitrite and the presence of hydrogen sulfide) that we found was in the bottom 50 m at station 107 (Fig. 1).

Resolving the seasonal variability in the ocean off Peru is difficult even for temperature alone (*14*). Consequently, some of the seemingly unusual conditions may have resulted from seasonal variability. For example, intensified denitrification over the shelf might be largely associated with seasonal changes, since episodes of complete denitrification have been observed in other years between February and April (*8, 10, 15*). Seasonal changes may also be partly responsible for the development of the intense offshore nitrite maximum (Figs. 2 and 3). This feature was associated with a seasonal shoaling of a density isopleth ($\sigma_t = 26.0$). It is difficult, however, to attribute all of the changes to the seasonal cycle. For example, the 26.0 σ_t surface in the region of the intense nitrite maximum was at the shallowest level ever observed (*8*), with a mean depth of 39 m; the historical average is 90 m. In addition, the deeper nitrite maximum found between 7° and 10°S has its only known antecedent in the 1975 data.

It is tempting to examine the hypothesis that a significant acceleration in the marine denitrification rate took place off Peru in 1985. The most detailed estimate (*2*) of the rate in the eastern tropical South Pacific suggested a total rate of 25×10^{12} g/year in an oxygen-deficient volume of 150×10^{12} m^3. We assumed a volume of 12×10^{12} m^3 for the deep nitrite maximum observed between 7° and 10°S. We used an average denitrification rate of 0.175 g of nitrogen per cubic meter per year, based on 25 observations of the activity of the respiratory electron transport system (ETS) and assumptions made in the past (*2, 16*) to arrive at a denitrification rate for this feature of 2×10^{12} g/year. Estimating denitrification rates in the shallower sites of intensified denitrification is more difficult because (i) a significant fraction of the ETS activity may arise from organisms that are not involved in

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