ABUNDANT EXPRESSION OF TYPE \( K^+ \) CHANNELS

A Marker for Lymphoproliferative Diseases?\(^1\)

STEPHAN GRISSMER, * MICHAEL D. CAHALAN, * AND K. GEORGE CHANDY**\(^2\)

From the *Department of Physiology and Biophysics and Division of Basic and Clinical Immunology, Department of Medicine, University of California, Irvine, CA 92717

Using the patch clamp whole-cell recording technique, we studied expression of \( K^+ \) channels in mAb-defined T cell subsets from diseased C3H-lpr/lpr and C3H-gld/gld mice and from healthy C3H-HeJ congenic controls. Both mutant mouse strains develop a lupus-like syndrome accompanied by hyperplasia of a functionally and phenotypically abnormal T cell subset. These defective cells, which are Thy-1.2+ CD4- CD8- B220+ F23.1+*, display an abundance of type I \( K^+ \) channels. Phenotypically equivalent lymph node T cells from normal C3H-HeJ mice, or young C3H-lpr/lpr mice before the onset of disease, do not display large numbers of type I \( K^+ \) channels. CD4+ CD8+ T cells (helper/inducer) from the mutant mice express a small number of type \( n^+ \) \( K^+ \) channels, and CD4- CD8+ T cells (suppressor/cytotoxic) show a low level of type \( l^+ \) or \( n^+ \) \( K^+ \) channels, as do their phenotypically equivalent counterparts in the normal mouse thymus. These results suggest that the abundant expression of type I \( K^+ \) channels is a marker for the defective lpr and gld T cell subset and may reflect the "abnormal" proliferative status of these cells.

At least three types of voltage-gated \( K^+ \) channels, termed \( n^+ \), \( n^- \), and \( l^+ \), are expressed by murine T cells (1-3). These \( K^+ \) channels can be distinguished on the basis of their pharmacology, kinetics, voltage dependence, and single-channel conductance (1-3). Normal murine resting lymph node T cells express small numbers (~10 to 20 channels/cell) of one of these types of \( K^+ \) channels, whereas lymph node-derived T cells activated by the mitogen, ConA, possess about 20 times more \( K^+ \) channels (4, 5). Recently, Lewis and Cahalan (2, 3) reported that CD4+ CD8+ thymocyte subsets (helper/inducer) express type \( n^+ \) \( K^+ \) channels (20 to 100/cell), whereas CD4- CD8+ thymocytes (suppressor/cytotoxic) display type \( l^+ \) or \( n^+ \) \( K^+ \) channels (20 to 200/cell). Thus, \( K^+ \) channel expression could be used as a marker to distinguish T cell subsets.

MRL-MpJ mice homozygous for the autosomal recessive mutation lpr (denotes lymphoproliferation) develop substantial lymphadenopathy and a disease resembling SLE (6). The proliferating cells in these hyperplastic organs express the TCR (7-10) and the Thy-1 marker, indicating T cell lineage (8, 11-13). However, unlike mature T cells, these cells do not express either the CD4 or the CD8 T cell differentiation markers (8, 13, 14), and they additionally express a pre-B cell marker, Ly-5 or B220 (8, 13). These cells have been suggested to represent an immature T cell subset. The lpr gene locus has been introduced into different murine genetic backgrounds (e.g. C3H-HeJ-lpr/lpr, C57Bl6-J-lpr/lpr) (15, 16). All mouse strains expressing the lpr mutation develop lupus accompanied by hyperplasia of T cells with the same unique cell surface phenotype (15). The chromosome location of the mutant gene lpr has not been mapped (17).

Another mutation, gld (generalized lymphoproliferative disease), also induces hyperplasia of T cells phenotypically and functionally identical to the expanded subset in MRL-MpJ-lpr/lpr mice (8, 17-19), along with lupus-like disease. The location of this mutant gene has been mapped to chromosome 1 approximately 10 centimorgan from the dominant loop tail gene lp (17).

We previously reported that the majority of lymph node T cells from diseased MRL-MpJ-lpr/lpr mice express a large number of type \( I \) \( K^+ \) channels (20). The lpr mutation is the first example of a genetic defect associated with an ion channel alteration in cells of the immune system. Several interesting questions arise from this observation. 1) Does the lpr mutation alone, or in combination with other genes, cause altered ion channel expression in lymphocytes? 2) Would other single-gene mutations that lead to autoimmune disease and lymphoid hyperplasia result in abnormal channel expression? 3) Is the abnormal channel expression in MRL-MpJ-lpr/lpr T cells correlated with a specific T cell phenotype? We have addressed these questions in the present investigation.

MATERIALS AND METHODS

Mice

C3H-HeJ, C3H-HeJ-lpr/lpr and C3H-HeJ-gld/gld mice were purchased from the Jackson Laboratory (Bar Harbor, ME). One C3H-HeJ mouse, seven C3H-HeJ-lpr/lpr mice, and three C3H-HeJ-gld/gld mice were examined in the studies described in this report. The three C3H-HeJ-gld/gld mice and three of the seven C3H-HeJ-lpr/lpr mice examined were >15 wk of age and demonstrated severe lymphadenopathy. One C3H-HeJ-lpr/lpr mouse was 12 wk old and did not
CD8- cells, still attached to the recording pipette. were counter-

Antibodies

PE- and FITC-conjugated anti-CD4 (Lyt-2) and anti-CD8 antibodies were purchased from Becton Dickinson (Mountain View, CA). Anti-Thy-1.2 and anti-B220 (RA3-1B2) were a generous gift from Dr. R. P. Shingarekov (Research Institute of Scripps Clinic, La Jolla, CA); the antibody reacts with all three members of the V8 family. Fluoresceinated goat anti-mouse IgG antibody (affinity purified and absorbed with rat Ig) and PE-conjugated goat anti-rat IgG (affinity purified and absorbed against mouse Ig) were purchased from Callag (Rupp and Hewman, Tustin, CA).

Separation of T Cells

Mice were killed, and single-cell suspensions were prepared from the thymus or lymph nodes. In previous experiments, we had observed that the $g_{\text{max}}$ values of T cells from lymph nodes and spleen were similar; therefore, in the present study, we pooled the cells obtained from thymic, inguinal, and axillary lymph nodes (20). T cells were enriched by passage through a nylon wool column.

Direct staining

Cells [2 x 10^6] were incubated with an appropriate dilution of anti-CD4-PE and either anti-CD8-FITC or anti-Thy-1.2- FITC for 30 min on ice, washed three times, and resuspended in 1 ml of RPMI 1640 medium containing 10% heat-inactivated FCS (HyClone, Logan, UT) and 2 mM L-glutamine. The stained cells were plated into glass chambers, and the major T cell subsets were then identified by epifluorescence microscopy. When anti-CD4 and anti-CD8 antibodies were used, three populations were evident: orange channel expression was then characterized in these cells. In most mammalian Ringer, incubated for a further 10 min with goat anti-mouse IgG-FITC, washed again with mammalian Ringer, and then three times with medium. They were then incubated with goat anti-rat IgG-PE (binds anti-CD4, anti-CD8, and anti-B220 antibodies), washed three times with medium. Next, the cells were incubated for 20 min with goat anti-Ig-PE (binds anti-CD4, anti-CD8, and anti-B220 antibodies), washed three times with medium, and then incubated for a further 20 min with goat anti-rat Ig-PE (binds anti-CD4, anti-CD8, and anti-B220 antibodies). The cells were then incubated for 20 min with goat anti-rat Ig-PE (binds anti-CD4, anti-CD8, and anti-B220 antibodies). The cells were washed three times with medium. The cells were then resuspended in 1 ml of medium. Only F23.1+ CD4-CD8-B220- cells (green cells) were voltage clamped. The remainder of the cells, which were orange, yellow, or unstained, were not examined. Contaminating B cells (surface Ig\(^{+}\) and B220\(^{+}\)) were excluded from the voltage clamp analyses because these cells would be yellow.

Electrophysiologic Experiments

After phenotypic identification by epifluorescence microscopy, single T cells were voltage clamped by using the whole-cell recording mode of the patch clamp technique. Details of the gigohm-seal voltage clamp technique used here are described elsewhere (22). All experiments were done at room temperature (22 to 26°C).

RESULTS

The lpr gene mutation causes altered ion channel expression in lymph node T cells, irrespective of the genetic strain of the mouse. C3H-lpr/lpr and C3H-ld mice develop a lupus-like disease along with lymphoproliferation of a Thy-1.2 CD4\(^{-}\)CD8\(^{-}\) TCR \(^{\text{B220}}\) T cell subset that is phenotypically identical to the abnormal cells in MRL-MpJ-lpr/lpr mice (8). Figure 1 shows K\(^{+}\) outward currents in the major T cell subsets defined by the CD4 and CD8 markers in C3H-lpr/lpr mice. In these experiments, the membrane potential of the cells was held at $-80$ mV, and then depolarizing pulses were applied to $+40$ mV. This protocol opens all the voltage-gated K\(^{+}\) channels in the

3 Abbreviations used in this paper: PE, phycoerythrin; TEA, tetraethylammonium; $g_{\text{max}}$, maximal K\(^{+}\) conductance.
cell. CD4+ CD8- cells (Fig. 1A) have small K+ currents that are half-blocked by 10 mM TEA (top) and are use dependent (bottom), indicating that they express small numbers of type n K+ channels. CD4- CD8+ cells (Fig. 1B) display small K+ currents that are half-blocked by 0.1 mM TEA (top) and are not use dependent (bottom), indicating that they express low levels of type l K+ channels. Some CD4- CD8+ cells also express small numbers of type n' K+ channels (currents not shown). The abnormal CD4- CD8- T cell subset (Fig. 1C) has large K+ currents that are half-blocked by 0.1 mM TEA (top) and are not use dependent (bottom), indicating that the defective cells abundantly express type l K+ channels.

Figure 2A shows the gsk,max in CD4- and CD8-defined T cell subsets from C3H-lpr/lpr mice. The abnormally proliferating CD4- CD8- T cells have a gsk,max of 5223 ± 565 pS (mean ± SEM; n = 22). Dividing the gsk,max by the reported single K+ channel conductance for type l (3) gives an estimate of approximately 200 type l K+ channels/cell, similar to the aberrant MRL-MpJ-lpr/lpr T cells (20). CD4+ CD8+ cells have a gsk,max of 145 ± 30 pS (mean ± SEM; n = 6), representing approximately eight type n K+ channels/cell. CD4+ CD8- cells have a gsk,max of 270 ± 77 pS (mean ± SEM; n = 8), representing an average of 6 to 12 type l or n' K+ channels/cell. Thus, abundant type l K+ channels are present in defective CD4- CD8- T cells from diseased C3H-lpr/lpr mice. In contrast, CD4+ CD8- (helper/inducer) and CD4- CD8+ (suppressor/cytotoxic) T cells from the lymph nodes of C3H-lpr/lpr mice express the same type and roughly the same numbers of K+ channels as their phenotypically identical counterparts in the normal thymus (2, 3).

Another single-gene mutation, gld, leads to autoimmune disease and proliferation of lymph node T cells abundantly expressing type l K+ channels. C3H-gld/gld develop both a lupus-like disease and the proliferation of a T cell subset phenotypically similar to that in lpr mice. Because the lpr and gld mutations produce the same disease, despite being non-allelic, it is conceivable that the abnormal T cells in gld mice have alterations of K+ channels similar or even identical to those in C3H-lpr/lpr/lpr cells. Figure 2B demonstrates that the aberrantly proliferating CD4+ CD8- T cells in C3H-gld/gld mice also have a gsk,max of 5092 ± 503 pS (mean ± SEM; n = 26), representing approximately 200 type l K+ channels/cell. C3H-gld/gld CD4+ CD8+ T cells have a gsk,max of 42 ± 3 pS (mean ± SEM; n = 5), representing approximately 2 to 3 type n K+ channels/cell. CD4+ CD8+ T cells have a gsk,max of 324 ± 98 pS (mean ± SEM; n = 5), indicating an average of 3 to 18 l or n' K+ channels/cell. Thus, the abnormal CD4+ CD8- T cells from sick C3H-gld/gld mice display a high level of type l K+ channels as do the defective MRL-MpJ-lpr/lpr T cells (20) and the C3H-lpr/lpr/lpr T cells (cf. Fig. 2A). On the other hand, CD4+ CD8- (helper/inducer) and CD4- CD8+ (suppressor/cytotoxic) T cells from the lymph nodes of these mice express a "normal" K+ channel phenotype.

Abundant type l K+ channels are exhibited by Thy-1.2+ CD4+ CD8+ B220+ Fcarr 1* lymph node-derived T cells from C3H-lpr/lpr and C3H-gld/gld mice. In Figure 2, A and B, we demonstrated that the aberrant CD4- CD8- T cell subset in C3H-lpr/lpr and C3H-gld/gld mice display many type l K+ channels. We examined these CD4- CD8- cells for the expression of Thy-1.2, B220, and...
Figure 2. Maximal $K^+$ conductance of CD4$^+$ CD8$^-$, CD4$^+$ CD8$^+$, and CD4$^-$ CD8$^+$ T cell subsets: A. Diseased C3H-lpr/lpr mice (>15 wk old); B. healthy CSH-lpr/lpr mice (2 to 5 wk old); C. CD4$^+$ CD8$^+$ T cells from normal C3H-HeJ mice (>15 wk old); D. CD4$^+$ CD8$^+$ T cells from healthy C3H-lpr/lpr (2 to 5 wk old). Maximal $K^+$ conductance reflects the number of $K^+$ channels per cell. The maximal $K^+$ conductance was measured as described in Materials and Methods.

Figure 3. Maximal $K^+$ conductance of Thy-1.2$^+$ CD4$^+$ and Thy-1.2$^+$ CD4$^-$ T cells in C3H-lpr/lpr mice. Maximal $K^+$ conductance reflects the number of $K^+$ channels per cell. The maximal $K^+$ conductance was measured as described in Materials and Methods.
tions of B220+ and F23.1+ thymocytes detected by epifluorescence microscopy would be similar to that reported with flow cytometry. B220+ cells were evident in roughly 1% (475 cells counted) of fresh thymocytes and in 2% (492 cells counted) of F23.1+ thymocytes also stained with anti-B220 antibody. In two experiments, F23.1+ cells were found in 2% (1130 cells counted) and 9% (407 cells counted) of normal thymocytes; F23.1 staining was dim and may account for the lower percentage in our test compared with reported results obtained with flow cytometric analysis.

A phenotypically similar lymph node T cell subset from normal C3H-HeJ congenic controls does not express large numbers of type I K+ channels. A B220+ thymocyte subset, otherwise phenotypically similar (Thy-1.2+ CD4- CD8+ F23.1+ J11D+) to the aberrant lpr and gld cells, has recently been identified in several murine strains (23-26). Because these cells acquire B220 on activation, they have been hypothesized to be a source of the abnormal lpr and gld cells (23). A phenotypically similar lymph node T cell subset has also recently been identified (23). It is conceivable that the abundant expression of type I K+ channels is another cell surface feature of this novel T cell subset. We therefore examined K+ channel expression on CD4- CD8- B220+ F23.1+ cells obtained from lymph nodes of normal C3H-HeJ congenic controls (see Materials and Methods). Figure 2C shows the gK,max of this subset. Unlike the defective lpr or gld cells (cf Fig. 2, A and B), the normal cells have a gK,max of 234 ± 69 pS (mean ± SEM; n = 12), representing approximately 12 channels/cell of either types n, n', or I K+ channels or a mixture of these K+ channels. Thus, the abundant expression of type I K+ channels may be a feature of the diseased T cell subset in lpr and gld mice.

A phenotypically similar T cell subset from the lymph nodes and thymus of young C3H-lpr/lpr mice, obtained before the onset of disease, does not express abundant type I K+ channels. Because the number of CD4+ CD8- cells increases with age in both C3H-lpr/lpr and C3H-gld/gld mice, we examined CD4+ CD8- lymphodeprived T cells from 2- to 5-wk-old C3H-lpr/lpr mice (n = 3) with no overt evidence of disease. Five CD4+ CD8- lymphodeprived T cell subsets from C3H-lpr/lpr and C3H-gld/gld mice (cf Fig. 2A and 3) as do phenotypically similar cells from diseased MRL-MpJ-lpr/lpr mice (20). Inasmuch as the diseased T cells from these two genetically distinct mice strains expressing the lpr mutation exhibit an abundance of type I K+ channels, the altered K+ channel phenotype is likely to be associated with the lpr mutation, irrespective of the genetic background of the mouse or the susceptibility of the strain to develop the lupus-like syndrome.

The gld mutation, although distinct from and nonallelic with the lpr mutation, induces hyperplasia of a phenotypically similar T cell subset (18, 19). Inasmuch as the proliferating T cells from C3H-gld/gld mice also display a high level of type I K' channels (Figs. 2A and 3) as do phenotypically similar cells from diseased MRL-MpJ-lpr/lpr mice (20). Inasmuch as the diseased T cells from these two genetically distinct mice strains expressing the lpr mutation exhibit an abundance of type I K+ channels, the altered K+ channel phenotype is likely to be associated with the lpr mutation, irrespective of the genetic background of the mouse or the susceptibility of the strain to develop the lupus-like syndrome.

A novel thymocyte subset (Thy-1.2+ CD4- CD8- J11D- TCR+) has recently been identified (23-26). Unlike the abnormal lpr and gld cells, these cells do not express B220, but a variable fraction of these cells acquire B220 after activation with anti-TCR antibody plus IL-1 (23). Since these cells are phenotypically similar to the abnormally proliferating lpr and gld T cells, this subset has been hypothesized to be a source for the defective cells (23). A phenotypically identical lymph node T cell subset has also been reported (23). Recently, McKinnon and Ceredig (27) reported that this thymocyte subset, identified as CD4+ CD8- J11D- thymocytes, expresses small K' currents (gK,max = 0.5 ± 0.3 nS) of uncertain type. In the present study, we demonstrate that the phenotype similar subset (CD4+ CD8- B220+ F23.1+) from the lymph nodes of healthy C3H-HeJ mice, displays a small number of K' channels of types n, n', or I. Furthermore, phenotypically similar cells from young
C3H-lpr/lpr mice tested before the onset of disease do not exhibit altered K’ channel expression. These data imply that the absent expression of type I K’ channels by the defective CD4+ CD8+ lpr and gld lymph node T cells is a feature of the disease. However, it is not clear whether the absence of type I K’ channels in the lpr and gld mice reflects a causal relationship between type I K’ channels and abnormal proliferation or whether it is a manifestation of the disease.

The defective lpr and gld T cells do not express the IL-2R nor do they produce IL-2, molecules normally expressed by lectin- and Ag-activated T cells (13, 19). Furthermore, lectin- and Ag-activated T cells display a large number of type n K’ channels (5, 20), in contrast to the abundance of type I K’ channels expressed by the diseased lpr and gld cells. Taken together, these data suggest that a proliferative signal, distinct from the normal lectin- and Ag-activated pathway, causes the hyperplasia of those defective T cells that abundantly express type I K’ channels.

Palicacios (28) reported that spleen cells from MRL-MpJ-Ipr/lpr mice constitutively produce an IL-3-like activity when tested on an IL-3-dependent pre-B cell line and suggested that this lymphokine may be responsible for the lymphoid hyperplasia in these mice. In contrast, Kelly and colleagues (29) did not confirm this result with spleen cells from MRL-MpJ-Ipr/lpr mice or three other mouse strains bearing the mutant lpr gene either by the bioassy or by measuring IL-3 mRNA. However, two recent reports by Ohta et al. (30, 31) indicate that, although lymphoid cells from MRL-MpJ-Ipr/lpr mice do not constitutively secrete an IL-3-like substance, sera from these diseased mice contain IgG with IL-3-like activity. B cell hybridomas that secrete mAb with IL-3-like activity have been established from sick MRL-MpJ-Ipr/lpr mice (31). Based on these data, the authors conclude that lymphoid hyperplasia in mice bearing the lpr mutation may result from stimulation by autoantibodies directed against the abnormal lymphocytes of CSH-lpr/lpr mice. The proliferation of the lymphoproliferating cells of the MRL-lpr/lpr mice are a parallel pathway of normal and abnormal T cell development. J. Immunol. 139:2200.

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