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Defect in ERK2 and p54^{JNK} Activation in Aging Mouse Splenocytes

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We have previously reported on a defect in both extracellular signal-regulated protein kinase (ERK) and c-jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) activation in splenocytes obtained from old rats. In order to investigate whether these effects are conserved across species, we have now used mouse splenocytes to measure the effect of aging on the activation of the same two MAPK families: ERK and JNK. Our results demonstrate that, as in rats, both MAPK signal transduction pathways are affected by aging in mice, indicating the existence of a further defect located downstream of the receptor-proximal events. Whereas ERK1 and p46^{JNK} activation were not significantly modified, the kinetics of both ERK2 and p54^{JNK} activation and inactivation were affected in splenocytes from old animals. Specifically, by analyzing the kinetics of activation and inactivation of these enzymes, we found a nearly 50% decrease in the fold of activation of both ERK2 and p54^{JNK}. These defects result in an overall diminution of enzyme activities without changes in the steady-state levels of relevant proteins. The impaired activity of these two MAPK pathways is likely to play a role in the reduced expression of interleukin-2 and diminished lymphoproliferation observed in old animals.

T-CELL activation and proliferation require both the T-antigen receptor and costimulatory molecules. The early events after T-cell stimulation lead to the activation of several protein tyrosine kinases (PTKs) of the src family, such as Lck and Fyn, as well as the zeta-chain-associated protein kinase ZAP-70 (1). These events result in the recruitment and phosphorylation of anchoring proteins, which indirectly couple to phospholipase C (PLC) activation, resulting in increased levels of intracellular calcium and activation of protein kinase C (PKC) (2). The activation of PTKs also leads to the recruitment of adaptor proteins such as Shc, growth factor receptor-bound protein 2 (Grb2), and son of sevenless (Sos) to the cytoplasmic membrane. Sos, a guanine exchange factor, induces Ras activation resulting in the activation (via *Raf*-1 and MEK) of the mitogen-activated protein kinase (MAPK) extracellular signal-regulated protein kinase (ERK) (3). It has been described that activation of the ERK pathway is required for interleukin-2 (IL-2) gene induction, and IL-2 is the major growth factor in T cells (4).

Evidence for the requirement of ERK activity for IL-2 gene expression comes from experiments in Jurkat cells, where overexpression of ERK isoforms leads to increased IL-2 gene expression, whereas overexpression of a dominant-negative mutant of *Raf*-1 results in diminished IL-2 expression (5). Furthermore, it has been shown that inhibition of the ERK pathway in T-cell clones challenged with antigen also results in a decrease in IL-2 mRNA levels (6).

Activation of T lymphocytes also requires the activity of another member of the serine/threonine MAPK family, c-jun N-terminal protein kinase/stress-activated protein kinase (JNK/SAPK) (1). Whereas ERK activation only requires the T-cell receptor (TCR) stimulus, JNK activation is synergistic upon costimulation of both the TCR and CD28 receptors (7). The JNKs are encoded by at least three separate genes, each of which can give rise to several isoforms by alternative splicing. JNK3 is specifically expressed in brain, and each one of the other genes, JNK1 and JNK2, may originate isoforms of 54 and 46 kDa (8). They are activated preferentially by cellular stress, but also by growth factors and G protein-coupled receptor agonists (9). The JNK pathway is distinct but parallel to the pathway responsible for activation of ERK (10,11). It is clear that the activation of both MAPK pathways is required for maximal IL-2 gene expression.

The immune response is compromised in aged individuals, and this occurs at several different levels (12–19). The most consistent change with increasing age is a decrease in the proliferative response of T cells in response to a variety of stimuli (12,13). Most but not all researchers also find a decrease in IL-2 production (reviewed in 1, 14, 16–19). Miller has argued that the disagreements in the literature are possibly the result of differences in the method of activation used (19). A series of studies suggest that age-related disturbances in signal transduction pathways might explain the diminished IL-2 gene expression. It has been shown that ty-

rosine phosphorylation of Shc, Fyn, and ZAP-70 are all diminished in anti-CD3-stimulated T cells from old mice (20) and humans (21). In contrast, in ConA-stimulated T cells from rats, an age-related defect was observed only on the activation of Lck and ZAP-70, but not Fyn (22). These results suggest the existence of a defect in the early steps of T-cell activation. Additional studies performed in aged murine and human T cells have shown an age-related impairment in the activation of both *Ras* and *Raf*-1 (23,24), again suggesting age-related impairments located upstream of these molecules within the pathway. Kinetic studies show that the age-related impairments represent reductions in both the level and duration of MAPK activation (25–30).

The early events in MAPK signal transduction can be circumvented through the use of phorbol 12-myristate 13-acetate (PMA) plus the Ca^{+2} ionophore (CaI) A23187, which together act as effective comitogens for some species of lymphocytes (31). It has been shown, however, that the age-related defect in immunoproliferation is still apparent when cells are stimulated with PMA plus CaI (32), and this suggests that a further defect in signaling might be located downstream of the TCR-proximal events. It is important to note, however, that although activation of total splenocytes by means of the TCR/CD3 complex results in specific activation of T lymphocytes, the use of PMA plus CaI is likely to activate other cell populations as well, including B cells (33). Indeed, it has been reported that stimulation of human B cells with either CaI or PMA alone can induce Tyr phosphorylation, as well as the enzymatic activation of ERK (34). Similarly, JNK activity is increased after both CD40 and B-cell receptor ligation (35). Using mouse splenocytes as a model, we have used PMA plus CaI to further investigate the existence of age-related defects in signal transduction pathways located downstream of the receptor-proximal defects already described. Studies on the effect of age on PMA plus CaI-induced activation of T cells have generally used proliferation as an end point. We chose to assess the changes directly in ERK and JNK MAP kinase activities in response to PMA plus CaI stimulation. In rats, we have observed an age-related decrease in these activities (28). We extend these results to the mouse model, and further we show here that the kinetics of activation of both pathways is affected by aging in mouse splenocytes. In addition, our results indicate that not all isoforms of these enzymes are equally affected by the aging process.

Methods

Mice

C57BL/6 male mice were used for all experiments. They were purchased from the National Institute on Aging colony of the Charles River Laboratory, and upon arrival, they were housed in our specific pathogen-free facility for at least 2 weeks before use. The mice received food and water ad libitum. The mean life span of male C57BL/6 mice is approximately 24 months. In this study, young or old refers to ages of 4–6 months or 24–26 months, respectively. The young animals are therefore sexually mature at the time of assay.

In all experiments, aliquots of the cells were used to assess proliferative capacity in response to 4 μg/ml ConA. All pools of splenocytes from old animals demonstrated a diminished proliferation in this assay, ranging between 35% and 62% of the level observed in matched samples from young animals (data not shown). This is in accordance with data from the literature (36–38).

Spleen Lymphocyte Isolation

Animals were sacrificed by cervical dislocation. The spleens from three animals, either young or old, were pooled and homogenized in a Dounce homogenizer in Roswell Park Memorial Institute (RPMI) 1640 (BioWhittaker, Walkersville, MD). The cell suspension was then carefully overlaid on Histopaque 1083 (Sigma, St. Louis, MO), centrifuged at 800 × *g* for 20 minutes at room temperature, and the splenic mononuclear cells were carefully collected from the media/Ficoll interphase and resuspended in RPMI 1640.

Cell Stimulation and Whole Cell Extract Preparation

After one wash in RPMI, the splenocytes were resuspended at 5×10^6 cells/ml in RPMI without serum, and cells were allowed to equilibrate at 37°C, 5% CO_2 for 1–2 hours. Before stimulation, 5% fetal bovine serum was added, and the cells were stimulated with PMA (50 ng/ml) plus the CaI A23187 (1 μg/ml) for the indicated times.

For whole cell extracts (WCE) to be prepared, harvested cells were suspended in lysis buffer as described by Li and colleagues (28), shaken at 4°C for 30 minutes, and cleared by centrifugation. The supernatant (WCE) was collected and protein concentration was measured by the Bradford assay as commercialized by BioRad (Hercules, CA).

Western Blot Analysis

Aliquots (30 μg) of WCE from each time point were used for a Western blot analysis. Samples were denatured by boiling in sodium dodecyl sulfate (SDS) loading buffer, reduced with 5% β-mercaptoethanol, and fractionated in 10% or 12.5% SDS-polyacrylamide gel electrophoresis mini gels. Proteins were then transferred to nitrocellulose filters (BA 85, Schleicher & Schuell, Keene, NH); the membranes were blocked with bovine serum albumin (BSA) buffer (1% BSA, 10 mM Tris, pH 7.5, 100 mM NaCl, 0.1% Tween 20) for 1 hour at room temperature and probed with horseradish peroxidase conjugated antiphosphotyrosine monoclonal antibody (Transduction Laboratories, Lexington, KY) for 1 hour at room temperature. After the samples were washed three times for 15 minutes in TNT buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 0.1% Tween 20), detection was performed by using the enhanced chemiluminescence (ECL) system from Amersham (Arlington Heights, IL), according to the instructions supplied by the manufacturer.

To measure steady-state levels of kinases, we used 30 μg of WCE of unstimulated mouse splenocytes prepared from four individual animals of each age. The protocol was analogous to the previous one, except that after the transfer, the membrane was blocked three times for 15 minutes with 5% nonfat dry milk (Carnation, Nestlé USA, Solon, OH) prepared in a buffer of 25 mM Tris, pH 7.5, 1 mM ethylenediamine tetra-acetic acid, and 140 mM NaCl (TEN buffer). Membranes were then probed with specific primary anti-

bodies (Transduction Laboratories) overnight at 4°C, followed by three washes of 20 minutes each in 3% nonfat milk in TEN. The blot was then exposed to peroxidase-conjugated goat antimouse IgG secondary antibody for approximately 1.5 hours at room temperature and then washed extensively in TEN buffer. Detection was performed by using ECL as before. Before reprobing with a new antibody, the blot was stripped by shaking for 30 minutes at 50°C in a solution containing 62 mM Tris (pH 6.7), 2% SDS, and 100 mM β-mercaptoethanol. Then the membrane was extensively washed in TEN buffer and reprobed with another antibody. As a normalization control, we probed the membrane with antiactin antibody (ICN; Biomedical Research Products, Costa Mesa, CA).

Western blot analysis with the antiactive ERK and antiactive JNK antibodies was performed exactly as suggested by the manufacturer (Promega, Madison, WI).

"In gel" Kinase Assays

Ten or 30 μg of WCE were used for the ERK and JNK kinase assays, respectively, according to the procedures described previously (28).

Data Acquisition and Analysis

Films from appropriately short exposure times were scanned and quantitated using the ImageQuaNT software analysis program (Molecular Dynamics, Sunnyvale, CA). The values represent the mean ± standard error obtained from three independent pools of splenocytes. The nonparametric Kruskal–Wallis test was used in the analysis of nonrelated samples (i.e., young versus old), whereas the Friedman and Quade test was used for related samples (i.e., time course experiments). For statistical significance, we considered as significant a value of $p < .05$.

Results

Spleen Cells Show Diminished Proliferation and an Impairment in Tyrosine Phosphorylation

In this study, we measured ERK and JNK activities in total unfractionated mouse splenocytes. These represent a complex population containing approximately 25–30% T cells, 60–65% B cells, and approximtely 5% macrophages and natural killer cells. In order to characterize the impairment in proliferation previously described in cells from aging subjects, we stimulated spleen cells obtained from young or old mice. In these experiments, we observed that spleen cells from older mice demonstrate an approximately 47% ($p < .05$) lower proliferative potential than cells obtained from young animals. Furthermore, and in agreement with previous studies, we observed an age-related decline in total tyrosine phosphorylation (33). Figure 1A shows the kinetics of tyrosine phosphorylation of proteins in the 40- to 60-kDa range of molecular weight in pools of mouse splenocytes treated for various times with PMA plus A23187. As shown in this figure, stimulation of cells obtained from young animals leads to a rapid and strong induction of phosphorylation of two proteins in the apparent molecular weight range of 42–44 kDa. This event is diminished in cells obtained from old animals. The apparent molecular weight of these species suggests that they might correspond to ERK2 and ERK1.

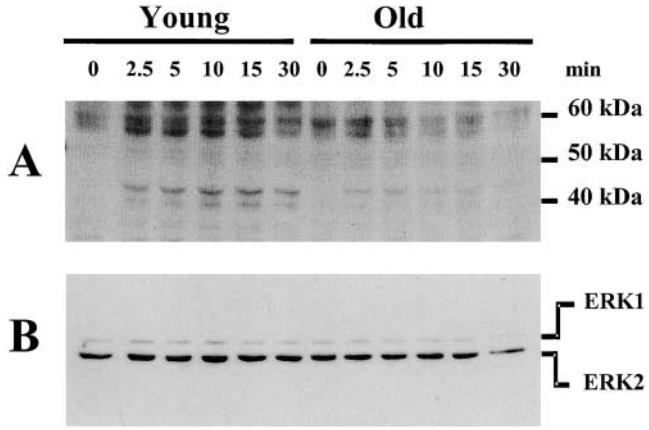

Figure 1. Protein tyrosine phosphorylation in response to phorbol 12-myristate 13-acetate plus calcium ionophore induction is diminished in splenocytes from old mice. Pools of splenocytes isolated from three young and three old C57BL/6 mice were induced with phorbol 12-myristate 13-acetate (50 ng/ml) plus A23187 (1 μg/ml) for the times indicated. Aliquots (30 μg) of the whole cell extract were electrophoresed on a 12.5% sodium dodecyl sulfate polyacrylamide gel and transferred to nitrocellulose membranes. The stimulation time (in minutes) is indicated. **A**: Immunodetection was performed with horseradish peroxidase conjugated anti-P-Tyr antibody. Binding was detected by an enhanced chemiluminescence system. **B**: Both extracellular signal-regulated protein kinase (ERK) isoforms (ERK1 and ERK2) were detected by Western blot analysis with anti-pan-ERK antibody, followed by horseradish peroxidase-conjugated secondary antibody.

ERK2, but not ERK1, Activity Is Impaired During Aging

Both the phosphotyrosine (P-Tyr) data and previous reports suggest that ERK activities might be impaired in splenocytes from old mice. We next tested directly the activation of ERK enzymatic activity upon splenocyte stimulation. ERK activity was measured by "in gel" kinase assays. A representative experiment is shown in Figure 2A. The results indicate that both ERK activities (ERK1 and ERK2) are quickly induced in response to PMA plus CaI in splenocytes isolated from animals of either age. However, ERK activation is diminished at all times in splenocytes isolated from old animals compared with that of younger counterparts.

As an independent measure of ERK activation, we analyzed the same samples by Western blot, using antiactive ERK antibodies (Figure 2B). The results obtained by this method are comparable with those obtained by the in gel kinase assay, and they indicate that the basal levels of ERK in splenocytes from old and young mice are similar. We conclude that ERK activation is impaired in splenocytes obtained from old mice compared with that of their young counterparts. The experiments indicate that phosphorylation and activation of both ERK isoforms occurs rapidly and remains elevated for at least 30 minutes.

Whereas the data presented, and that in the literature, indicate that ERK activity is rapidly induced in lymphocytes, we next tested the kinetics of disappearance of ERK activity

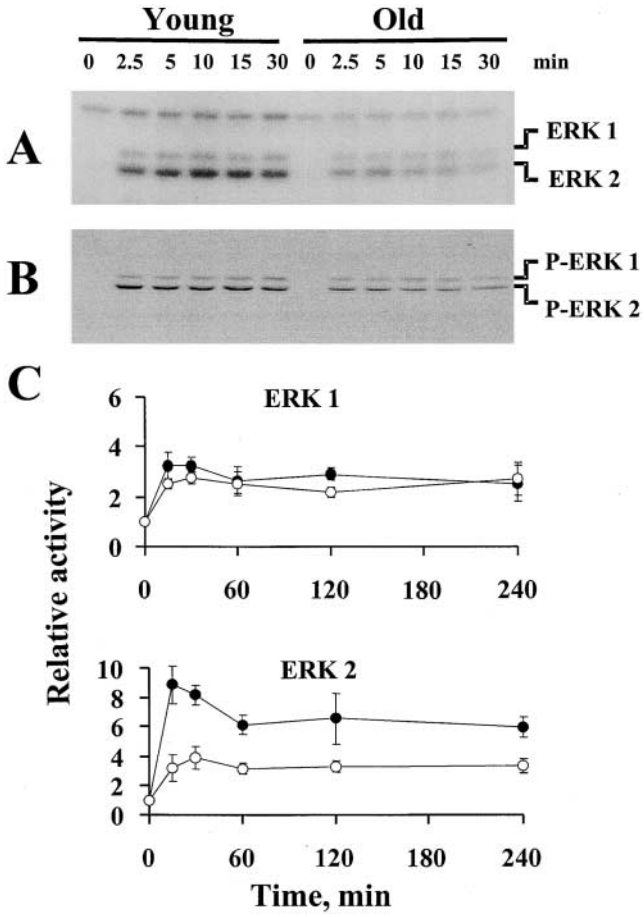

Figure 2. Extracellular signal-regulated protein kinase (ERK) activation in response to phorbol 12-myristate 13-acetate plus calcium ionophore induction is diminished in splenocytes from old mice. **A**: Aliquots of whole cell extract (10 μg) from the same experiment shown in Figure 1 were used to measure ERK activity by the in gel kinase assay. **B**: ERK activity was measured by Western blot analysis of the same membrane, using an anti-active ERK antibody. In both cases, the results are representative of three independent experiments. The phosphorylated forms of ERK are indicated (P-ERK1 and P-ERK2). **C**: Activation of ERK2 but not ERK1 is impaired in aging splenocytes. Pools of splenocytes (three animals/pool of each age) were induced as in Figure 1 for the indicated times. Aliquots of whole cell extract (10 μg) were used to measure ERK activity by the in gel kinase assay. The bands corresponding to the 44-kDa ERK1 and the 42-kDa ERK2 were quantitated from three independent experiments. Values are mean ± standard deviation, and values have been standardized against the basal level of activity at time zero; ●, young; ○, old. Similar results were obtained if the raw data (not standardized) are plotted (data not shown).

for periods up to 4 hours. The results (Figure 2C) indicate that the induction of both ERK activities in response to PMA plus CaI is quite persistent and easily detectable above basal levels for at least 4 hours in animals of both age groups. In contrast, T-cell stimulation with either anti-CD3 or anti-CD3 plus PMA leads to ERK activity that starts to decline by 10–15 minutes in both humans and mice (23,38). Our results further indicate that age does not significantly affect the degree of activation of ERK1 in splenocytes exposed to PMA plus CaI. In contrast, compared with that of younger counterparts, ERK2 activity is significantly diminished in splenocytes from old animals at all times examined, with an average diminution of 49 ± 3% ($p < .01$). Furthermore, as in the case of ERK1, ERK2 activity in young animals reaches a maximum at 15 minutes and then declines slowly. It should be noted that in these cells, ERK2 activity is more predominant than ERK1 (Figures 2A and 2B).

JNK Activities Are Also Differentially Impaired During Aging

Because JNK, but not ERK, activity requires the synergistic effects of both PMA and A23187, or, in vivo, the costimulation of both the TCR and CD28 pathways (7), we wanted to explore whether JNK activation is also affected by age. To test this possibility, we measured JNK activity both by in gel kinase assay and antiactive JNK antibodies. Figure 3A shows a representative example of the kinetics of JNK activation, as measured by Western blot using antiactive JNK antibodies. The results indicate that, like ERK activity, JNK activity is induced in response to PMA plus A23187, and this activity is diminished during aging. Similar results were obtained using the in gel kinase assay for JNK. Therefore, our results suggest that the defect we observe in JNK activation most likely represents a reduction in signal transduction within the pathway. Figure 3B indicates the equal loading of the gel used in Figure 3A, as demonstrated by the similar intensity of the $p46^{JNK}$ protein signal.

As in the case of ERK, we next tested the full kinetics of JNK activation and deactivation for periods of up to 4 hours. The results (Figure 3C) indicate that $p54^{JNK}$ activation is slightly but reproducibly impaired at all times in splenocytes from old animals compared with that of younger counterparts. This impairment occurs both in the kinetics and at the level of fold induction. As shown in Figure 3C, $p54^{JNK}$ activity from spleen cells obtained from young animals returned to basal levels after 240 minutes of treatment ($p < .05$). In contrast, in spleen cells from old mice, this recovery occurs earlier, at 120 minutes ($p < .05$). These results suggest that aged mice have a diminished level of $p54^{JNK}$ activity. Careful analysis of $p46^{JNK}$ activity, which is decreased in old animals by an average of 29 ± 2%, indicated that this effect is not statistically significant. We therefore conclude that activation of $p54^{JNK}$ is more impaired than $p46^{JNK}$ activity.

The Steady-State Level of the Kinases Is Not Affected by Aging

It is generally believed that the activity of MAPK signal transduction pathways is controlled primarily at the level of phosphorylation and dephosphorylation (39). However, it is clear that changes in the abundance of these proteins could also affect the activity of the pathways (40). Therefore, we have used Western blot analysis to establish whether or not the steady-state level of different MAPK is affected by age in mouse splenocytes. For this, we have used total cellular extracts prepared from unstimulated splenocytes from four individual mice of each age group. The membrane was probed sequentially with anti-ERK1, ERK2, JNK1, and p38 antibodies. The results presented in Figure 4 indicate that there are no significant age-dependent changes in the steady-

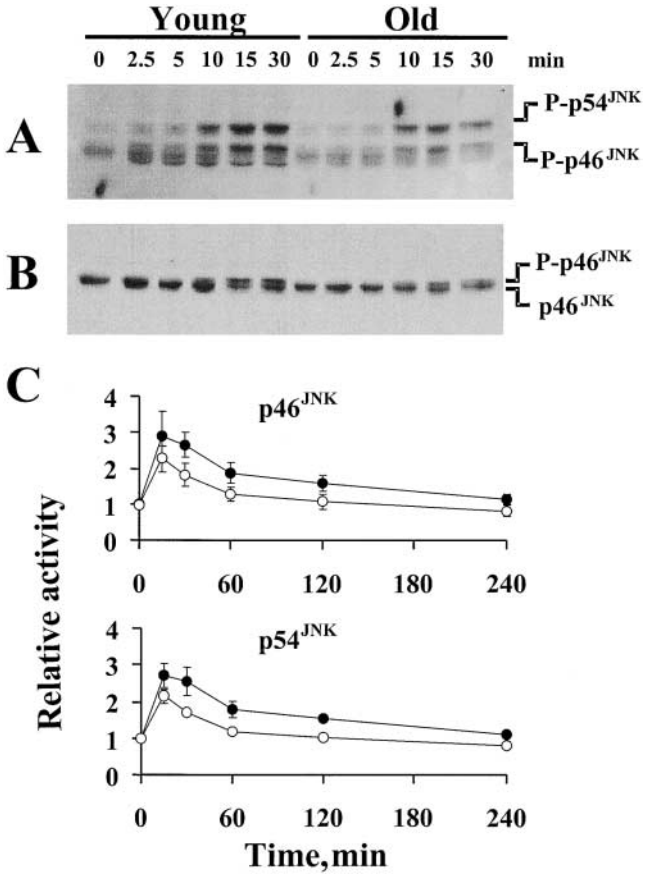

Figure 3. The c-jun N-terminal protein kinase (JNK) activation in response to phorbol 12-myristate 13-acetate plus calcium ionophore induction is diminished in splenocytes from old mice. **A**: JNK activity was measured by Western blot analysis of the same membranes used in Figure 2, using anti-active JNK antibody. The phosphorylated forms of JNK are indicated (p46 and p54). **B**: The same membrane was probed with anti-JNK1 antibody, followed by horseradish peroxidase-conjugated secondary antibody. **C**: Activation of $p54^{JNK}$ but not $p46^{JNK}$ is impaired in aging splenocytes. JNK activity was measured in triplicate by in gel kinase assays (three animals/pool of each age). The bands corresponding to the 54-kDa $p54^{JNK}$ and the 46-kDa $p46^{JNK}$ were quantitated. Values are mean ± standard deviation, and they have been standardized against the basal level of activity at time zero; ●, young; ○, old. Similar results were obtained if the raw data (not standardized) are plotted (data not shown).

state level of any of these proteins. Thus we conclude that the changes in activity cannot be ascribed to variations in the steady-state level of the kinases, but rather, either to their degree of activation by upstream effectors, or to their inactivation by relevant phosphatases. The steady-state levels of these other relevant molecules have yet to be investigated.

Discussion

The MAPK family plays a crucial role in the activation of both T and B cells (33). ERK activity, and particularly ERK2, has been shown to be required for T-cell proliferation, and the costimulatory signals provided by activation of the CD28 receptor have been shown to be required for both maximal JNK activity and IL-2 production. In the present

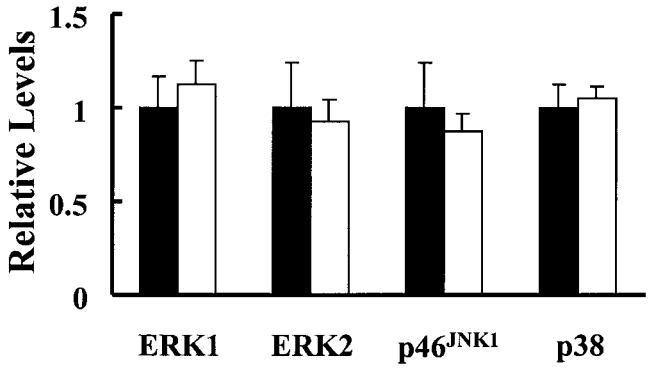

Figure 4. The steady-state level of mitogen-activated protein kinase proteins is not affected by age in mouse spleen lymphocytes. As a way to determine the steady-state levels of different signal transduction-related proteins, aliquots (30 μg) of whole cell extract obtained from unstimulated splenocytes obtained from individual young and old mice ($n = 4$ for each age) were subjected to Western blot analysis as described in the Methods section. The membrane was probed sequentially with anti-extracellular signal-related protein kinase (ERK)1, ERK2, c-jun N-terminal protein kinase (JNK)1, and p38 antibodies. The values have been standardized against β-actin (from antiactin antibody), whose levels do not change significantly as a function of age, and data are presented relative to the mean level observed in splenocytes from young animals, which were arbitrarily given a value of 1. The values given represent the mean ± standard deviation for young (black bars) and old (open bars) animals.

study, we bypassed the early events in either TCR-directed or B-cell-receptor-directed signal transduction by using PMA plus CaI as a stimulus and have analyzed both ERK and JNK activation in unfractionated splenocytes from young and old mice. Thus, the use of PMA plus CaI to stimulate lymphocyte proliferation provides an independent approach to the question of whether alterations in the generation of internal signals are critical to immunosenescence.

In agreement with previous reports, we found a decreased proliferative capacity in spleen cells obtained from old animals than in those obtained from young animals. Furthermore, our results indicate that splenocytes from old mice present a defect within the two MAPK pathways studied (ERK and JNK), which is located downstream of receptor activation. In the case of ERK, we observed a marked diminution of nearly 50% in ERK2 activity, whereas ERK1 activity showed no decrease, but only a little significant decline in maximal activation. Although this impairment in ERK activation was observed in unfractionated spleen cells, our results are in agreement with the impairment in ERK activity observed in mouse $CD4^+$ T cells (19; for review see 24). Furthermore, these results are also consistent with other reports showing an age-related decrease in ERK activity in other cell types, such as hepatocytes (41–44).

A decrease in JNK activation as shown here has also been described in human (23,27) and murine T cells (29,30). In this pathway we also found a series of defects, including a reduction in the activity of $p54^{JNK}$, with a much smaller (and not statistically significant) reduction in the activity of $p46^{JNK}$. For both isoforms, we observed an early deactivation in comparison with young animals. Our results are in agreement with data previously reported by Kirk and Miller (29,30). They detected a reduction in JNK activation that

was specifically observed after proliferative stimuli, but not after stress-induced activation of lymphocytes (30). Our results suggest that the different isoforms of ERK and JNK might be activated and inactivated by means of different mechanisms. By using knock-out animals, Kane and colleagues (1) and Pawelec and Solana (45) recently described that each MAPK isoform might have a different function during T-cell development (1,45).

Although there is considerable disagreement with respect to *Ras* activation in T cells from old mice and rats (23,24,30), our protocol avoids this step in the pathway by bypassing *Ras* activation. Under these conditions, our results demonstrate a defect located downstream, possibly at the level of ERK itself. A similar conclusion can be drawn for the JNK pathway. Alternatively, the defect might be controlled by age-related changes in the levels and activity of MAPK phosphatases, and we are currently exploring this possibility. An interesting point is the analogy that exists between previous and our observations and T-cell anergy (26,28,46). Indeed, it has been reported that incomplete stimulation of T cells leads to a state of unresponsiveness (anergy), characterized by a decrease in both *Ras* and ERK activities, without affecting the protein levels of these proteins (47,48).

By having the full complement of splenic cells, our model recapitulates rather faithfully the conditions found in vivo during lymphocyte activation. However, a clear problem arises from the use of relatively nonspecific inducers such as PMA, plus A23187 treatment leads to a similar level of activation in both cases. The effect of aging on both of these main populations of lymphocytes has been well studied. There is currently little evidence for a dramatic change in the number or type of B lymphocytes with aging in either human blood (49) or mouse spleen (50), but changes in the proportion of B-cell subsets have been reported (reviewed in 19). In the case of T cells, the main age-related change described is a dramatic shift in the proportion of naive and memory T cells within the $CD4^+$ and $CD8^+$ pools (51). This has been described in both mice and humans. Recently, it has been reported that the age-associated accumulation of memory T cells could be the result of chronic activation of PKCα, which should promote the generation of memory T cells and inhibit the elimination of previously generated memory cells (52). Interestingly, it has been observed that naive and memory $CD4^+$ T cells from young mice are equally able to convert ERK2 to its slower migrating form in response to anti-CD3 stimulation (20).

In summary, although the defects we have observed are likely to represent changes in all or most of the cell types present in our mixed cell population, a dramatic functional and phenotypic change during aging has been observed primarily in the T-cell compartment (13,49). For this reason, we believe that the defects in signal transduction we have observed are more physiologically relevant in these cells. In contrast, it is clear that proliferation and differentiation of both cell types is critical to effective humoral and cell-mediated immune responsiveness to pathogens and perhaps tumors. Therefore, we expect our studies to be of high significance to our further understanding of immunosenescence and the medically relevant immunodeficiency in the old.

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