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Age-Associated Decline in cdk1 Activity Delays Cell Cycle Progression of Human T Lymphocytes

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Despite the repeatedly observed impaired proliferative response of T lymphocytes from aged donors, the precise molecular basis underlying such a defect is still poorly understood. The aim of this study was to determine whether cyclin-dependent kinase 1 (cdk1), a serine-threonine kinase required for entry into mitosis, is implicated in this age-associated dysregulation of the cell cycle. T lymphocytes derived from young and elderly donors were blocked in S phase by hydroxyurea after a 48-h activation by anti-CD3 Abs. Under these experimental conditions, only the cells that were already located beyond the S phase were able to complete the cell cycle, decreasing their DNA content from 4n to 2n chromosomes. Using this procedure, a delay in the accomplishment of mitosis could be observed in cells from elderly individuals, as evidenced by propidium iodide staining. In this age group, only a minimal cdk1 activity could be immunoprecipitated from cells sorted in G2/M after nocodazole block. The decrease in cdk1 activity observed in T lymphocytes from aged donors could be accounted for by at least three mechanisms: 1) a failure of these cells to express a sufficient amount of cdk1, 2) a reduced level of the associated cyclin B1, and 3) an incomplete dephosphorylation of the kinase on tyrosine. This low cdk1 activity is likely to postpone the progression through the G2/M transition and participates in the dysfunction of the cell cycle during the process of aging. The Journal of Immunology, 1998, 161: 5203–5209.

During the process of senescence, the control of cell division is altered. The disturbances in the regulation of proliferative homeostasis that accompany aging play a significant role in the genesis of several age-related disorders, including immune deficiency. In fact, the central event responsible for most of the various aspects of age-associated immune dysfunction is a significant decrease in the proliferative potential of T lymphocytes. The proliferative response of T lymphocytes to mitogenic lectins (1–5), to mAbs directed against components of the TCR complex (6), and to soluble Ags (7) is decreased significantly in aged humans and rodents. Cytokine analyses using [3H]thy-midine uptake (8–10), flow cytometry (8–10), 5-bromo-2′-deoxyuridine (BrdUrd) incorporation (10, 11), and limiting dilution assays (12–14) have almost invariably shown an age-related decline in the number of T cells able to leave the G0 state upon activation to enter the cell cycle. In addition, T cells that manage to leave G0 after activation may not be able to complete successive cycles since age-associated alterations in cell cycle transition rates (10), length of cycles (11), and number of cycles successfully completed (8, 11, 15) have been observed. The molecular mechanisms involved in the difficulty to progress through the different phases of the cell cycle during the process of aging are still largely unknown.

Current understanding of the mechanisms implicated in the control of cell cycle attributes a key regulatory role to complexes between cyclin-dependent kinases (cdk) and cyclin subunits. Among the various cdk driving the eukaryotic cell cycle, the role of p34cdc2 (cdk1) is relatively well understood. cdk1 is required for entry into M phase (16). Its activity is regulated by association with type A and type B cyclins (17) and by phosphorylation-dephosphorylation reactions (18). Moreover, the cdk1 system appears to be connected to the IL-2-triggered transmembrane signaling cascade (19).

The scientific literature documenting the role played by cdk1 and related kinases in the age-associated modification of proliferative homeostasis concerns only senescent fibroblasts. Human diploid fibroblasts that have lost their proliferative capacity after in vitro aging fail to generate a significant quantity of transcripts from the cdk1, cdk2, cdk4, cyclin A, B, and D3 genes (20–22). Surprisingly, the same senescent cells express high levels of cyclin D1 and E mRNA (22) and accumulate large amounts of inactive cyclin E-cdk2 and cyclin D1-cdk2 complexes (23). We are unaware of any publication concerned with the cdk system in relation to in vivo aging.

In the present study, we investigated the role played by cdk1 in the proliferative defect of T lymphocytes derived from elderly individuals. We show that a significant decrease in cdk1 activity is associated with a partial inability of cells derived from aged donors to complete mitosis and reenter the next cell cycle. The age-related decrease in cdk1 activity could in turn be explained by a low expression of cdk, a reduced level of cdk1/cyclin B1 complexes, and an incomplete dephosphorylation of the kinase on tyrosine residues.

Materials and Methods

Cell preparation, cell activation, and cell cycle arrest

Heparinized blood was obtained from 13 young donors (<35 yr old) and from 13 volunteers >80 yr of age who met the admission criteria for immunogerontologic studies (SENEUR protocol) (24). Mononuclear cells were separated by centrifugation on a Ficoll-Hypaque cushion (Pharmacia, Duebendorf, Switzerland) and depleted twice from adherent cells by a 1-h incubation at 37°C in complete medium (RPMI 1640 containing 10% FCS,
25 mM HEPES, and 100 µg/ml gentamicin). This technique yielded cell preparations that were consistently >85% CD3⁺ as quantitated by flow cytometry.

T lymphocytes were activated by anti-CD3 mAb immobilized on a solid matrix. Briefly, 6-well and 96-well culture plates (Costar, Dottikon, Switzerland) were first coated with sheep anti-mouse IgG (Cappel, Pfalzikon, Switzerland) as described previously (25) and then exposed to anti-CD3 mAb containing supernatant from OKT3 hybridoma (American Type Culture Collection, Manassas, VA) grown in protein-free medium (Life Technologies, Basel, Switzerland). T cells were adjusted at 10⁶/ml in complete medium and cultured at 37°C in the anti-CD3-coated plates.

T lymphocytes to be fixed in S phase were exposed to 1 mM hydroxyurea (50 mM) for 24 h at the end of a 48-h culture. Cells to be blocked at the G2/M transition were incubated with 0.1 µg/ml nocodazole (Sigma, Buchs, Switzerland) (27) for 24 h before termination of a 72-h culture.

**Cell cycle analysis and cell sorting**

The percentage of cells in G0/G1, S, and G2/M phases was determined by cytofluorometric analysis following propidium iodide (PI) staining (28). For PI staining, 10⁶ cells were suspended in 100 µl of PBS without Ca²⁺ and Mg²⁺, and 200 µl of 95% ethanol was added while vortexing. The cells were fixed at 4°C for ≥ 1 h. washed in PBS, and resuspended in 250 µl of PBS containing 12.5 µg of RNase. Incubation was continued at 37°C for 30 min before staining cellular DNA with 250 µl of a PI solution (50 µg/ml in PBS) for 30 min at room temperature.

To determine the proportion of anti-CD3-activated cells able to undergo mitosis, T cells engaged in DNA synthesis were first blocked by 1 mM hydroxyurea as described previously. These experimental conditions, the G2 compartment could not be replenished by cells engaged in DNA replication. However, T cells already located beyond the S phase were insensitive to hydroxyurea and were still able to proceed through the M phase. A cell aliquot was taken for PI staining before and after a 10-h hydroxyurea block. The rate of decrement of 4n chromosome-containing T lymphocytes, quantified by cytofluorometry, was interpreted as an indication of their mitotic capacity.

Purification of live T lymphocyte populations located in defined phases of the cell cycle (G0/G1, S, G2/M) was achieved by cell sorting of nocodazole-blocked cultures, after vital DNA staining by Hoechst 33 342 (29). For each experiment, an aliquot of the sorted cells was reanalyzed for DNA content to verify the purity of the selected cell population. The 2n and 4n chromosome-containing lymphocytes were in excess of 97 and 92%, respectively, in the sorted cell preparations. These analyses were all performed using a FACSscan cytofluorograph equipped with Lysys 2 software (Becton Dickinson, Basel, Switzerland).

**Immunoprecipitation**

Cellular lysates were prepared by suspending no more than 5 × 10⁶ activated or 10⁶ resting cells in 100 µl of lysis buffer (1 mM sodium orthovanadate, 25 mM MOPS, pH 7.2, 15 mM MgCl₂, 15 mM EGTA, 1 mM DTT, 1 mM sodium orthovanadate, 10 mM β-glycerophosphate, 15 mM 4-nitrophosphophate, and 0.1% Tween-20) containing 0.1 mM PMSF, 2 µg/ml leupeptin, 2 µg/ml aprotinin, and 2 µg/ml E-64 (all protease inhibitors are from Sigma). The cells were disrupted by sonication and extracted at 4°C for 30 min. Lysates were clarified by centrifugation at 13,000 × g for 5 min, and the amount of protein contained in the supernatants was determined using the Protein Assay ESL kit supplied by Boehringer Mannheim (Rotkreuz, Switzerland).

The supernatants (50 µg of protein) were then precipitated for 2 h at 4°C with 25 µl of protein G-agarose beads (Calbiochem, Luzern, Switzerland) precoated with saturating amounts of rabbit antiserum prepared against the seven carboxyl-terminal amino acids of human p34cdc2 (30) and obtained from Dr. A. A. Nordin (Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, MD). Alternatively, polyclonal Abs to cyclin B1 (Upstate Biotechnology, Lake Placid, NY) were used in a similar fashion to immunoprecipitate cyclin-cdk complexes. The protein G-agarose was collected by centrifugation (2000 × g for 3 min), and the beads were washed with 1 ml of lysis buffer.

**Immune complex kinase assay**

After three additional washes in kinase assay buffer (identical with lysis buffer, except that EGTA was 5 mM and Tween-20 was omitted), the immunoprecipitated proteins on beads were assayed for histone H1 kinase activity as reported previously (31). Briefly, the beads were suspended in 50 µl of kinase assay buffer containing 2.5 µg histone H1 (Boehringer Mannheim), 40 µM ATP, and 2 µCi [γ-32P]ATP (3000 Ci/mmol) (Amersham, Zurich, Switzerland). After incubation for 30 min at 30°C with occasional mixing, the samples were boiled in polyacrylamide gel sample buffer containing SDS and separated by electrophoresis. Phosphorylated histone H1 was visualized by autoradiography. Films were scanned using a laser densitometer equipped with ImageQuant Software (Molecular Dynamics, Krefeld, Germany).

**Protein analysis**

For detection of cdk1 and cyclin B1 from sorted cells at various stages of the cell cycle, 50 µg of cell lysates or the immune complexes recovered on protein-G agarose were denatured by boiling in Laemmli sample buffer, separated on 12% SDS-polyacrylamide gels (32), and electrotransferred to Immobilon-P membranes (Millipore, Volketswil, Switzerland). To avoid cross-reactions and interference in detection, proteins that were immunoprecipitated with rabbit Abs were probed with murine Abs and vice versa.

The phosphorylated form of cdk1 was recognized by phosphospecific (Typ+) anti-ckd1 Abs (New England Biolabs, Beverly, MA, Detection was achieved using enhanced chemiluminescence (Amersham), according to the manufacturer’s instructions. Lumigrams were scanned as described above.

**Data analysis**

Each experiment was performed simultaneously on paired samples derived from young and elderly subjects. The effect of age was assessed by a pairwise comparison of the observed values using the Wilcoxon signed-rank test.

**Results**

Anti-CD3-induced mitosis is delayed in T lymphocytes derived from elderly humans

We first verified that the group of 13 elderly donors selected for these studies presented with the classical age-related cell cycle dysfunction. Table I shows that, irrespective of the duration of anti-CD3 activation, the T lymphocytes derived from the old age group have a limited capacity to enter the cell cycle and progress through its successive stages.

To determine whether anti-CD3-activated T lymphocytes derived from elderly subjects were specifically restrained in their ability to proceed through the G2/M transition, we monitored the rate of depletion of the G2 compartment under conditions that did not allow its continuous feeding by cells that recently completed DNA synthesis. The replenishment of the G2 compartment by cells, as visualized by PI staining, in activated T lymphocyte populations from young and elderly individuals, before (t = 48 h) and after the indicated period of activation, an aliquot of T lymphocytes derived from each of the 13 couples of young and elderly donors used throughout these studies were stained with PI and submitted to cytofluorometric analysis. Data are presented as the percentage of cells located at the various stages of the cell cycle.

<table>
<thead>
<tr>
<th>Activation Time</th>
<th>G0/G1 Young</th>
<th>G0/G1 Old</th>
<th>S Young</th>
<th>S Old</th>
<th>G2/M Young</th>
<th>G2/M Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD3 24 h</td>
<td>97</td>
<td>99</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>α-CD3 48 h</td>
<td>83 ± 4</td>
<td>90 ± 3</td>
<td>8 ± 4</td>
<td>5 ± 2</td>
<td>9 ± 2</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>α-CD3 72 h</td>
<td>67 ± 6</td>
<td>80 ± 5</td>
<td>16 ± 4</td>
<td>11 ± 4</td>
<td>17 ± 2</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>α-CD3 72 h + noc</td>
<td>58 ± 5</td>
<td>76 ± 6</td>
<td>15 ± 2</td>
<td>10 ± 4</td>
<td>27 ± 5</td>
<td>14 ± 5</td>
</tr>
</tbody>
</table>

α-CD3 72 h + noc: after the indicated period of activation, an aliquot of T lymphocytes derived from each of the 13 couples of young and elderly donors used throughout these studies was stained with PI and submitted to cytofluorometric analysis. Data are presented as the percentage of cells located at the various stages of the cell cycle.
after \((t = 58\) h\) a 10-h block by hydroxyurea. The same experiment was repeated in 13 paired samples from both age groups (Fig. 1B). An average 60% decrease in the number of G\(_2\) T lymphocytes was observed in the cultures derived from the young controls, whereas only 40% of these cells underwent mitosis in the aged group \((p, 0.02)\).

The delayed mitosis of activated T lymphocytes from elderly individuals is associated with a decreased cdk1 activity

Because cdk1 activity is critical for the initiation of mitosis (16), we explored the possibility that the apparent reluctance of anti-CD3-activated T lymphocytes from elderly humans to enter M phase is related to a dysfunction of cdk1. To eliminate the possible interference due to cells located in G\(_0\), G\(_1\), or S phase and thus devoid of specific kinase activity, cdk1 activity was assessed in T lymphocytes selected in G\(_2\). To facilitate the process of cell sorting, it was necessary to enlarge the G\(_2\)/M compartment by blocking the G\(_2\)/M transition with nocodazole for the last 24 h of the culture. After vital DNA staining and subsequent sorting, the 2n and 4n chromosome-containing T lymphocytes were processed, as described above, for determination of their cdk1 activity. Histone H1 kinase activity immunoprecipitated from unselected and from 2n and 4n chromosome-containing T cells is shown in Fig. 2A. Because cdk1 activity is restricted to a short period preceding mitosis (33), no kinase activity could be detected in sorted 2n cells. In contrast, an important cdk1 activity was observed in unselected cells as well as in 4n-sorted cells. The kinase activity was logically increased in the selected cell population since it was entirely composed of cells arrested at the G\(_2\)/M border, whereas the unselected population contained a mixture of cells at various stages of the cycle. A marked decrease in cdk1 activity was observed in the unselected cells from aged individuals. The age-related difference in cdk1 activity remained evident in purified G\(_2\) cells. The same experiment was conducted in 10 parallel samples from young and old donors (Fig. 2B). In a pairwise comparison with the young controls, a significant decline in cdk1 activity was found in unselected \((p < 0.05)\) as well as in selected G\(_2\) T lymphocytes \((p < 0.02)\) from the elderly.

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** Cytofluorometric analysis of T lymphocytes able to undergo the G\(_2\)/M transition in young and elderly individuals. Anti-CD3-activated T cells were blocked in S phase, for 10 h, by 1 mM hydroxyurea added 48 h after the initiation of the culture. A cell aliquot was processed for PI staining before and after the S block. The decrement of hydroxyurea-insensitive G\(_2\) T cells that had already completed their DNA synthesis before the S block was monitored by flow cytometry. A. Representative analysis of 4n chromosome-containing T lymphocytes before \((t = 48\) h\) and after \((t = 58\) h\) hydroxyurea block. B. Percentage of decrease in 4n T lymphocytes during the 10-h S block in cell preparations derived from 13 young and 13 elderly donors. Each point represents the determination corresponding to one subject, and the horizontal bar indicates the median for each set of individual values.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** cdk1 activity in T lymphocytes from young and elderly individuals, selected in defined phases of the cell cycle. Anti-CD3-activated T cells were blocked at the G\(_2\)/M transition by the addition of 0.1 \(\mu\)g/ml nocodazole, 24 h before termination of a 72-h culture. The 2n and 4n chromosome-containing T lymphocytes were then sorted after vital DNA staining by Hoechst 33342 and processed for determination of cdk1 activity. A. Representative autoradiography showing the histone H1 kinase activity immunoprecipitated from unselected and from purified 2n and 4n T cells. B. Cumulative results expressed in arbitrary OD units determined by autoradiography scanning and obtained in 10 pairs of individuals from both age groups.
Activated T lymphocytes from elderly donors express a low level of cdk1 that could not be restored to normal by IL-2 supplementation

Because cdk1 is ultimately induced by cell activation (19) and because this process is altered with aging (34), we examined the eventuality that the age-related decrease in cdk1 activity was due to a low expression of the protein. As for histone H1 kinase activity, the level of expression of cdk1 was analyzed in unselected, 2n- and 4n-sorted T lymphocytes derived from anti-CD3-activated, nocodazole-blocked cultures from young and elderly individuals. The quantity of cdk1 detected by immunoblotting in the lysates of unselected T lymphocytes from the aged was markedly decreased by comparison with the level observed in similar cells from young controls (Fig. 3A). This age-related difference in cdk1 expression remained noticeable in sorted 4n cells, whereas purified 2n cells, as expected, did not synthesize any significant amount of the protein. cdk1 immunoblottings were repeated in 10 paired samples from young and aged donors (Fig. 3B). In a pairwise comparison with the younger subjects, cdk1 expression was found depressed significantly (p < 0.02) in unselected cells as well as in sorted 4n cells derived from the aged individuals.

Because cdk1 expression depends upon the availability of IL-2 (19) and because IL-2 production classically declines with age (35), we studied the effect of IL-2 supplementation on the expression of the kinase. As shown in Fig. 4, the addition of 1 μM exogenous rIL-2 at the initiation of the cultures from both young and elderly subjects did not result in a modification of cdk1 synthesis (A) or enzymatic activity (B).

The expression of cyclin B1 as well as the formation of cdk1/cyclin B1 complexes are decreased with aging

Aside from the low expression of cdk1, a deficient production of the regulatory cyclin B1 and/or a defective association with the kinase may result in an inadequate activation of cdk1. We therefore verified the level of cyclin B1 expression and its association with cdk1 as a function of age. Total cdk1 and total cyclin B1 were immunoprecipitated and detected by Abs of the same specificity, whereas cdk1/cyclin B1 complexes were immunoprecipitated by either anti-cyclin B1 or anti-cdk1 polyclonal Abs and detected with the reciprocal mAbs. These immunoprecipitations were performed on lysates derived from nocodazole-blocked, sorted 4n T lymphocytes. The same procedure was applied to 10 paired samples from both age groups. Fig. 5A shows such a representative experiment.

In Fig. 5B, the total cdk1 immunoprecipitated and detected by anti-cdk1 Abs in the cells from elderly individuals averaged 35% of the amount recovered from the cells of young donors. When compared with the total quantity of cyclin B1 immunoprecipitated from the cells of young individuals, the level of cyclin B1 detected in the T lymphocytes from the elderly was also decreased by 25%. Irrespective of the procedure used for immunoprecipitation and detection (i.e., immunoprecipitation by anti-cdk1 and detection by anti-cyclin B1 or vice versa), the level of cyclin B1/cdk1 complexes was diminished by 50% in the 4n-sorted cells from the aged as compared with similar cells from the young.

cdk1 is incompletely dephosphorylated in G2/M lymphocytes isolated from elderly persons

Along with cyclin association, phosphorylation-dephosphorylation reactions regulate cdk1 activity. Complete dephosphorylation at tyrosine 15 results in maximal activation of the kinase in G2/M cells (18). To verify whether the loss of cdk1 activity with age was related to a defective dephosphorylation of the kinase, we compared in both age groups the degree of phosphorylation of cdk1 in sorted 4n T lymphocytes using Abs that detect only the phospho-tyrosyl form of cdk1 (anti-(Tyr15) cdk1 Abs). Fig. 6A shows a typical tyrosine phosphorylation time course of cdk1 in unsorted anti-CD3-activated T lymphocytes from young adults. Phosphorylation at tyrosine 15 increases to reach a peak at 48 h, then decreases at 72 h, allowing, at that time, optimal expression of cdk1 activity and progression of cells through the G2/M transition. In Fig. 6B, cdk1 was immunoprecipitated from sorted 4n cells obtained 48 h and 72 h after activation. Detection by anti-(Tyr15) cdk1 Abs reveals a persistent phosphorylation of cdk1 at tyrosine 15 at both time points in T lymphocytes derived from the elderly, whereas a major decrease in tyrosine-specific phosphorylation is observed in the cells from the young controls. Similar results were obtained on a series of 10 individuals of both age groups (p < 0.02).
DNA replication (i.e., located in G2) was monitored by flow cytometry. Because inhibition of DNA synthesis by hydroxyurea is sensitive to cell cycle synchronization agents (37) prompted us to use a different approach. After an initial activation by anti-CD3 mAbs that allowed a significant proportion of the cells to progress through the different phases of the first cycle, the S phase was arrested in 10 paired samples.

Discussion

In the classical model used to study proliferative senescence, cells that have been induced to age in vitro by multiple rounds of replication become nondividing after a final arrest at the G2/M transition (36). In cells freshly isolated from senescent organisms, however, other blocks have been detected at different phases of the cell cycle (8–11, 15). For instance, Kubbies et al. (10) reported a cell cycle arrest in G2 in primary culture of PHA-stimulated lymphocytes derived from elderly humans. Using BrdUrd-Hoechst flow cytometry, they showed that ~10% of the activated cells from the aged donors were blocked in G2, whereas none of the cells from the young control was arrested in this compartment.

We were able to confirm the G2 arrest in anti-CD3-activated lymphocytes derived from aged individuals. In nocodazole-blocked, sorted 4n cells, total cdk1 and total cyclin B1 were immunoprecipitated and detected by Abs of the same specificity. In the same cell preparations, cdk1/cyclin B1 complexes were immunoprecipitated by either anti-cyclin B1 or anti-cdk1 polyclonal Abs and detected with the reciprocal mAbs. A, Representative immunodetections of p62 cyclin B1 and p34\(^{cdk1}\) in anti-cyclin B1 and anti-cyclin B1 immunoprecipitates. B, Cumulative results of total cyclin B1, total cdk1, and cdk1/cyclin B1 expressed in arbitrary OD values derived from lumigraphs scanning and obtained in 10 paired samples.

![Detection of cyclin B1, cdk1, and cdk1/cyclin B1 complexes in purified 4n T lymphocytes from young and elderly individuals.](image)

**FIGURE 5.** Detection of cyclin B1, cdk1, and cdk1/cyclin B1 complexes in purified 4n T lymphocytes from young and elderly individuals. In nocodazole-blocked, sorted 4n cells, total cdk1 and total cyclin B1 were immunoprecipitated and detected by Abs of the same specificity. In the same cell preparations, cdk1/cyclin B1 complexes were immunoprecipitated by either anti-cyclin B1 or anti-cdk1 polyclonal Abs and detected with the reciprocal mAbs. A, Representative immunodetections of p62 cyclin B1 and p34\(^{cdk1}\) in anti-cyclin B1 and anti-cyclin B1 immunoprecipitates. B, Cumulative results of total cyclin B1, total cdk1, and cdk1/cyclin B1 expressed in arbitrary OD values derived from lumigraphs scanning and obtained in 10 paired samples.

Although other molecular mechanisms may be involved in cell cycle regulation at the G2/M restriction point (38), entry into mitosis is essentially controlled by the activation of cdk1 (16). It is therefore conceivable that the low cdk1 activity detected in T lymphocytes isolated from the aged persons is responsible for the partial inability of these cells to complete the G2/M transition. As a result of the various cell cycle blocks and delays mentioned above (8–11, 15), the proportion of cells located in G2, G1, or S phase varies between individuals of different age. Because these cells are totally devoid of specific cdk1 activity, they indeed influence the degree of histone H1 kinase activity retrieved from a normalized quantity of protein. To eliminate this possible interference, it was therefore necessary to determine the level of cdk1 activity in purified G2 cells. The effect of such a cell selection appears clearly in Fig. 2. The fact that cdk1 activity immunoprecipitated from sorted G2 cells remains significantly lower in the elderly donors indicates that this age-related decrement is not merely the consequence of upstream cell cycle blocks, but represents an intrinsic defect of cells that manage to reach the G2 phase.

The same reasoning applies to the analysis of the intracellular content of cdk1. When we examined the possibility that the age-associated decrease in cdk1 activity was related to a low expression of the protein, cdk1 level was also measured in purified G2 cells. Because cdk1 is expressed only 30 h after stimulation and is not detected in G0 cells (33), it was indeed essential to avoid contamination by cells located in earlier phases of the cell cycle. The low amount of cdk1 detected in G2 lymphocytes undoubtedly affects its total kinase activity.

In a previous publication, we demonstrated that cdk1 expression depends upon the availability of IL-2 (19). Other studies showed that the increase in cdk1 activity is also IL-2-dependent.
(39). Because the classical age-related defect in IL-2 production (35) may interfere with the optimal function of cdk1, we attempted to boost both the expression and the activity of the kinase recovered in T lymphocytes from the elderly by supplementing the cultures with exogenous IL-2. The addition of saturating amount of rIL-2 modifies neither the level of expression of p34\(^{cdk1}\) nor its enzymatic activity. These results suggest that the age-related decrease in IL-2 production is not the limiting factor responsible for the decreased cdk1 expression. It is also conceivable that a simultaneous defect in IL-2R expression in cells from elderly individuals (35) prevents adequate signaling by exogenous rIL-2.

cdk1 activity is also regulated by its association with the mitotic cyclin B1. Because cyclin function is primarily controlled by changes in its own level (40), we verified the concentration of cyclin B1 in sorted 4n cells that had been blocked at the G2/M transition by nocodazole. At a state of progression through cell cycle that corresponds to a maximal cellular content of cyclin B1 (41), cells from the elderly still displayed a low level of the mitotic cyclin B1 (42). Therefore a binding defect due to an age-related alteration of the cyclin box cannot be ruled out, the reduced concentration of both cdk1 and cyclin B1 may account for the low level of cdk1/cyclin B1 complexes observed in the cells derived from the elderly.

In addition to cyclin binding, complete cdk1 activation requires abrupt dephosphorylation of Thr\(^{14}\) and Tyr\(^{15}\) at the end of the G2 phase (18). The availability of a phosphospecific Ab able to detect cdk1 only when phosphorylated at Tyr\(^{15}\) allowed us to compare the tyrosine phosphorylation status of the kinase in cells from young and old individuals, selected in early and late G2 phase. At a time when most G2 T lymphocytes from the young controls undergo tyrosine dephosphorylation, cells from the elderly group remain heavily phosphorylated. This observation suggests that the activity of cdk2, a dual-specificity phosphatase responsible for the dephosphorylation of Thr\(^{14}\) and Tyr\(^{15}\) (43), is also impaired with aging.

These results indicate that alteration of various molecular mechanisms underlies the age-related decline in cdk1 activity and the associated delay in the accomplishment of mitosis. Because the same mechanisms (synthesis/degradation, phosphor- ylation/dephosphorylation, complex formation) are implicated in the regulation of other cdks, it is probable that similar age-dependent modifications also affect the activity of these kinases at different phases of the cell cycle and participate in the overall loss of proliferative potential classically associated with the process of senescence.

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