clusion has been supported by the complete amino acid sequence of rhodopsin (*22*). If the nonhelical content of rhodopsin is taken into account, these helices should be oriented on average less than 30° from the membrane normal (*21*). We estimate that an average change of more than 5° would result in more than a 1 percent absorption change in the amide I region and conclude that 5° is the limit of orientational change of rhodopsin α-helices relative to the membrane on bleaching to meta II. In addition, a conformational change that resulted in a significant alteration of the secondary structure of rhodopsin would result in a much larger difference than we observed. Such a conformational change is also inconsistent with the results of ultraviolet absorption and circular dichroism measurements (*23*).

In conclusion, FTIR difference spectroscopy can provide detailed information about molecular changes in photoreceptor membrane. The effects of blocking rhodopsin bleaching at other intermediates such as meta I and the effects of deuterium-hydrogen exchange in the protein and chromophore will be reported subsequently. Further studies with chemically modified and reconstituted rhodopsin should be useful for identifying specific groups and their role in visual transduction. The techniques used here should also be applicable to other membrane-based systems, for instance, to study the active transport of calcium.

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27. Supported by grants from the National Science Foundation, the National Institutes of Health, the Whitaker Foundation, and an established investigatorship of the American Heart Association (to K.J.R.).

25 August 1982; revised 26 November 1982

Increased Sensitivity of Lymphocytes from People over 65 to Cell Cycle Arrest and Chromosomal Damage

Abstract. *Flow cytometry revealed that, in the presence of tritiated thymidine, a greater percentage of phytohemagglutinin-stimulated lymphocytes from old human donors were arrested in the G_2 or M phase than were cells from young donors. Furthermore, lymphocytes from old donors showed significantly more chromosomal damage than did lymphocytes from young donors. Lymphocyte cultures from old or young donors not exposed to tritiated thymidine had the same percentage of cycling lymphocytes in G_2 or M, although the number of lymphocytes stimulated by phytohemagglutinin to enter the cell cycle was significantly lower in cultures from old donors. Thus, the impaired incorporation of tritiated thymidine by phytohemagglutinin-exposed lymphocytes from old humans reflects both an impaired proliferative response to phytohemagglutinin and an increased sensitivity to the radiobiological effects of tritiated thymidine.*

When lymphocytes from aged humans and experimental animals are incubated with phytohemagglutinin (PHA), they incorporate less [^{3}H]thymidine than do lymphocytes from young donors (*1–3*). This has been attributed to an impaired proliferative capacity of lymphocytes from old donors, resulting from a paucity of mitogen-responsive T lymphocytes and from a failure of mitogen-responsive T lymphocytes to divide repeatedly in culture (*4, 5*).

Pollack *et al.* (*6*) reported that the use of [^{3}H]thymidine incorporation to assess lymphocyte proliferation in culture may be flawed. Using flow cytometry, these investigators found that the percentage of PHA-stimulated human lymphocytes in phase G_2 or M was significantly increased by exposure to [^{3}H]thymidine. This arrest of the cell cycle was secondary to the radiobiological effect of [^{3}H]thymidine. Neither tritiated water (which is not concentrated in the nucleus) nor thymidine-induced cell cycle arrest. It was suggested that the nucleus is the site of damage caused by [^{3}H]thymidine. The inability of cells to repair DNA damage induced by [^{3}H]thymidine may have resulted in the accumulation of lymphocytes in the G_2 or M phase.

Since cells from old donors are impaired in their capacity to repair DNA (*7*), it is possible that the reduced incorporation of [^{3}H]thymidine by lymphocytes from old as compared to young subjects is due at least in part to an increased sensitivity of lymphocytes from old donors to the radiobiological effects of [^{3}H]thymidine. We therefore determined the sensitivity of lympho-

Table 1. Cytogenetic analysis of PHA-stimulated lymphocytes exposed to [^{3}H]thymidine. Chromosomal preparations were prepared by adding Colcemid (0.05 μg/ml) to the cultures 1 hour before harvest. Ten milliliters of 0.075*M* KCl was added to each of the cell pellets, which were then incubated for 8 minutes at 37°C, fixed in methanol and glacial acetic acid (3:1), and washed three times in fixative. The final cell suspensions were dropped onto wet slides. Slides were stained with 3 percent Giemsa and coded for blind analysis. Metaphase figures derived from PHA-stimulated whole blood cultures exposed to [^{3}H]thymidine (0.1 μCi/ml) were analyzed. Cells were scored as abnormal if they contained exchange figures, chromosome breaks, or chromosome fragments.

Subject	Number of metaphase figures	
	Abnormal	Normal
Old		
1	17	8
2	14	11
3	10	3
Mean	13.67	7.33
Young		
1	9	16
2	9	16
3	10	15
Mean	9.33	15.66

cytes from young and old humans to cell cycle arrest and chromosomal damage induced by [^{3}H]thymidine.

Mononuclear cells were prepared from heparinized venous blood obtained from healthy volunteers of various ages (*8*). The proportion of monocytes in these preparations ranged from 15 to 23 percent. There were no significant differences in the percentage of monocytes, T lymphocytes, or B lymphocytes among the preparations.

Lymphocytes from ten young donors (ages 20 to 30) and ten old donors (over age 65) were cultured in the presence of PHA for 96 hours. One microcurie of [^{3}H]thymidine (specific activity, 1.9 Ci/mmole) was added to some cultures during the final 24 hours of the incubation period. Incorporation of [^{3}H]thymidine by lymphocytes from the older subjects (mean radioactivity per culture, 48.3 $\times$ 10^3 count/min) was approximately 50 percent of that incorporated by lymphocytes from the younger subjects (89.9 $\times$ 10^3 count/min) ($P < .001$, Student's *t*-test).

Flow cytometry was used to measure the DNA and RNA content of individual lymphocytes. The relative quantity of cellular DNA and RNA was used to distingush cycling cells from quiescent or dead cells (*9*). The proportion of cycling lymphocytes in G_2 or M in cultures from ten young donors was 16 percent, comparable to that in cultures from ten old donors (15.6 percent) (Fig. 1). In the presence of [^{3}H]thymidine, significantly higher percentages of cells in G_2 or M were found in cultures from old (40 percent) and young (25.8 percent) donors. The percentage of lymphocytes arrested in G_2 or M was significantly greater ($P < .002$) in cultures from old donors. Thus, while cell cycle arrest was induced by [^{3}H]thymidine in cells from all donors, lymphocytes from old donors appeared to be more sensitive to this effect.

Chromosomal morphology was also examined in lymphocytes cultured with PHA in the presence or absence of [^{3}H]thymidine. The incidence of abnormal metaphase figures in the absence of [^{3}H]thymidine was less than 1 percent in lymphocytes from old or young donors. A low dose of [^{3}H]thymidine, 0.1 μCi/ml, was chosen to test the sensitivity of cells to damage because higher doses caused chromosomal damage in all metaphase cells. Twenty-five metaphase cells in lymphocyte cultures from each of three young and two old donors were analyzed. In a third culture from an old donor it was possible to score only 13 metaphase cells. Lymphocyte cultures from old and young donors showed chromosomal abberations in 67 and 37 percent of the metaphase figures, respectively ($P < .002$). Thymidine had no effect on cell cycle progression or chromosomal structure in lymphocytes from young or old donors.

These results confirm the reports that [^{3}H]thymidine not only arrests the cell cycle but also causes chromosomal damage in lymphocytes cultured with PHA (*6, 10*). More important, they show that lymphocytes from old donors are more susceptible to the radiobiological effect of [^{3}H]thymidine than lymphocytes from young donors.

Incorporation of [^{3}H]thymidine has been used frequently to measure the proliferative response of lymphocytes. A number of investigators using this technique have suggested that the proliferative response of lymphocytes from old donors is impaired in culture with plant lectins (*1–3*). Studies of lymphocyte proliferation in the absence of [^{3}H]thymidine have supported the conclusions of studies in which [^{3}H]thymidine was used (*4*). Using flow cytometry in the absence of [^{3}H]thymidine, we found that significantly more lymphocytes from old donors remained in G_0 after a 48-hour incubation with PHA (50 percent) than did lymphocytes from young donors (34 percent) ($P < .002$). Thus, our results indicate that the reduced amount of [^{3}H]thymidine incorporated by lymphocytes from old as compared to young persons is due to both an impaired proliferative response to PHA and an increased sensitivity to [^{3}H]thymidine-induced arrest of the cell cycle.

Pollack *et al.* (*6*) suggested that cell cycle arrest in lymphocytes cultured with PHA and exposed to [^{3}H]thymidine for 18 hours results from radiation damage to DNA following the incorporation of [^{3}H]thymidine into nucleic acid. Chromosomal damage found in our studies supports this thesis. The increased sensitivity of lymphocytes from old donors was indicated by the increased number of lymphocytes arrested in G_2 or M and the increased percentage of chromosomally damaged metaphase figures. This, however, does not totally explain the lower [^{3}H]thymidine incorporation by lymphocytes from old humans. In the first place, flow cytometry in the absence of [^{3}H]thymidine showed that fewer lymphocytes from old donors enter

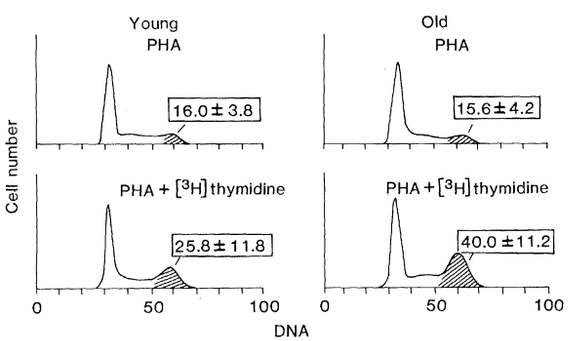

Fig. 1. Distribution of cellular DNA content in cycling PHA-stimulated lymphocytes from young and old donors in the presence or absence of [^{3}H]thymidine during the final 24 hours of culture. Shown are the results of a representative experiment comparing a young and an old donor. The shaded areas represent cells in the G_2 or M phase of the cell cycle. The boxed values give the mean percentage (± standard deviation) of cells in G_2 or M, as determined by a study of ten old and ten young donors. Diluted blood was layered over Lymphoprep and centrifuged at 400*g* for 20 minutes at 20°C. Mononuclear cells removed from the interface were washed three times with Hanks balanced salt solution, collected by centrifugation, and resuspended at a concentration of 2 $\times$ 10^6 cells per milliliter in complete culture medium (RPMI-1640 containing 15 percent heat-inactivated fetal bovine serum with 2 m*M* glutamine, 100 U of penicillin per milliliter, and 100 μg of streptomycin per milliliter; Gibco). Triplicate cultures were established in sterile round-bottom plates with multiple wells (Linbro 15-MRC-96-TC, Flow) containing 0.2 ml of the mononuclear cell preparation with or without purified PHA (5 μg/ml; Burroughs Wellcome) for 96 hours. One microcurie of [^{3}H]thymidine (specific activity, 1.9 Ci/mmole; Amersham) was added to each culture well during the last 24 hours of culture. The cells were then transferred to glass fiber filter paper with a Titertek cell harvester (Flow), placed in minivials with scintillant (Liquiscint, National Diagnostics), and counted. Replicate cultures were analyzed by flow cytometry for intracellular RNA and DNA content and for chromosomal figures. Cells (1 $\times$ 10^5 to 4 $\times$ 10^5) were suspended in complete culture medium and mixed with 0.4 ml of 0.08*N* HCl, 0.15*N* NaCl, and 0.1 percent Triton X-100 (Sigma) at 4°C. The cells were stained 30 seconds after the detergent treatment by the addition of 1.2 ml of a solution containing 0.2*M* Na_2HPO_4, 0.1*M* citric acid buffer (*p*H 6.0), 1m*M* EDTA-Na, 0.15*N* NaCl, and acridine orange (6 μg/ml; Chromatographically Purified, Polysciences, Inc.) (*12, 13*). Fluorescence of individual cells was measured in a FC200 Cytofluorograf (Ortho Instruments) interfaced to a Data General Nova 1220 minicomputer. The red (F > 600) and green (F530) fluorescence emissions generated by an argon-ion laser at 488 nm were separated optically and the integrated values of the pulses quantitated by separate photomultipliers. Background fluorescence was automatically subtracted. The number of lymphocytes in G_0, G_1, S, G_2, and M was determined on the basis of cellular RNA and DNA content (*9, 14*).

the cell cycle. In the second place, when a 20-minute exposure (which does not arrest the cell cycle) was used, lymphocytes from old donors incorporated less [^{3}H]thymidine than did lymphocytes from young donors; but this impairment was only 20 percent, as compared to the 46 percent impairment observed after a 24-hour exposure (*11*).

The effect of [^{3}H]thymidine on the progression of lymphocytes through the cell cycle and on chromosomal damage offers a new and potentially important probe of cell proliferation and the chromosomal stability of dividing cells in culture. The perturbation of cell cycle progression induced by [^{3}H]thymidine incorporation may be more pronounced in lymphocytes from old donors because of an increased sensitivity to radiation damage or a reduced capacity of lymphocytes from old individuals to repair DNA damage. The impaired proliferative response of lymphocytes from subjects with various diseases, as assessed by [^{3}H]thymidine incorporation, may therefore represent impaired activation of quiescent lymphocytes or increased sensitivity of the cells to the radiobiological effects of [^{3}H]thymidine.

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16 August 1982; revised 1 November 1982

H-2 Antigen Class: Effect on Mouse Islet Allograft Rejection

Abstract. *Rejection of mouse pancreatic islet allografts occurred in a high percentage of donor recipient combinations identical for H-2I–region antigens and differing at H-2K and H-2K + H-2D without I-region disparities. The results suggest that disparities in major histocompatibility complex antigens of class I (H-2K and H-2D) alone are capable of eliciting islet allograft rejection, and that lack of a stimulus from class II (I-region) alloantigens does not ensure permanent islet allograft survival.*

Prolonged survival of murine pancreatic islet allografts transplanted across a major histocompatibility barrier (H-2K + H-2I + H-2D disparate) was reported by Faustman *et al.* (*1*) after isolated islets were treated with donor-specific antiserum to I-region associated (Ia) antigens and complement. Loss of the Ia stimulus presumably led to prolonged islet allograft survival. Pancreatic islet β cells do not express class II (I-region) determinants, but do express class I (H-2K and H-2D) antigens (*2*). Protocols such as culture of islets (*3*) or treatment of islets with antiserum to lymphocytes (*4*) which lead to prolonged survival of islet allografts have been postulated to do so because they eliminate Ia-bearing lymphoid or other passenger cells within the islets that are necessary to initiate islet allograft rejection.

Evidence from studies in vitro indicates that Ia$^+$ cells are necessary for stimulation in the mixed lymphocyte reaction (MLR) regardless of whether the alloantigen differences involve either H-2K or Ia (*5*) determinants, and that Ia$^+$ stimulator cells are necessary in primary MLR's against both H-2K and H-2D gene products (*6*). Islet transplantation between congenic mice with disparities at the H-2K or H-2K + H-2D regions but with identical I-region loci can thus be used to test the requirement for allo Ia$^+$ cells to initiate an immune response to an allograft.

Previously, variable results were obtained in intrasplenic islet allograft experiments in which congenic strains differing at isolated I and S subregions of the H-2 complex were used (*7*). To define better the isolated contributions of class I and class II antigens we tested the effects of H-2K, H-2D, H-2K + H-2D, and H-2I-region disparities on islet allograft rejection in B10 congenic mice raised in the mouse colony of the Immunobiology Research Center at the University of Minnesota.

Recipient mice were made diabetic by intraperitoneal injection of streptozotocin (200 mg per kilogram of body weight). Only mice of the same sex as the donors, with nonfasting plasma glucose levels of more than 400 mg/dl, were used as recipients.

Islets were isolated by collagenase digestion as described (*8*). Briefly, for each transplant, intact pancreata were rapidly excised from seven overnight-fasted mice, cut into four pieces, and placed 2 pancreata per vial, into 8 ml of collage-

Table 1. Donor-recipient strain combinations and duration of islet allograft function after transplantation in diabetic mice according to isolated H-2 differences.

Donor strain	Recipient strain	H-2 disparity	Day islets rejected by individual mice	Time mice followed with continuing islet function (days)	Number functioning > 100 days (%)
B10.AQR	B10.AQR	None		> 110, > 110 > 130, > 190 > 190	100
B10.T(6R)	B10.AQR	I	21	> 100, > 100, > 100 > 100, > 100, > 100 > 100, > 100, > 100 > 110, > 100, > 110	92
B10.AQR	B10.A	K	8, 11, 13, 17, 19, 23, 23, 25	> 100, > 100, > 110	27
B10.A(2R)	B10.A	D	10	> 100, > 100, > 100 > 100, > 110, > 110 > 110, > 110	89
B10.AQR	B10.M(17R)	K + D	10,12, 17, 20, 25	> 100, > 110	29
B10.S	B10.G	K + I + D	12, 13, 13, 14, 18, 24, 26, 40	> 100, > 100	20