

AGING IN MAN

Linear Increase of a Novel T Cell Subset  
Defined by Antiganglioside Monoclonal Antibody 3G5

BY STEVEN L. RABINOWE,\* RAMESH C. NAYAK,\* KLAUS KRISCH,†  
KATHERINE L. GEORGE,\* AND GEORGE S. EISENBARTH\*

*From the \*Department of Medicine, Brigham and Women's Hospital, Section of Immunology,  
Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts 02115; and the*

*†Universitat Wien, Wien, Austria A-1090*

Despite multiple functional alterations of T cells with advancing age, standard mAb-defined T cell subsets are remarkably constant from youth to old age (1). In this communication we describe a novel "resting" human T cell subset defined by mAb 3G5. mAb 3G5 was originally produced after the immunization of C57BL6 × BALB/c mice with fetal rat brain (2). This cytotoxic IgM $\kappa$  mAb reacts with a ganglioside(s) expressed on the surface of a series of hormone-secreting cells, including pancreatic islets, a subset of anterior pituitary cells, and adrenal medullary cells (2).

Studies defining the relationship of this T cell subset to aging in man are described in this report.

Materials and Methods

*T Cell Studies.* To determine the percentage of 3G5<sup>+</sup> T cells in the peripheral blood and the possible existence of a relationship to aging we isolated T cells from the circulation of 21 normal individuals (ages 7–84) and determined the percentage of peripheral T cells reacting with mAbs 3G5, OKT4 (helper), and OKT8 (suppressor/cytotoxic) using a flow cytometer (3). In addition, we assessed the percent 3G5<sup>+</sup> T cells in nine pairs of monozygotic twins (ages 11–58). Heparinized venous blood was collected and the mononuclear cells separated by Ficoll-Hypaque density centrifugation. Purified T cells were obtained by incubating the mononuclear cells with neuraminidase-treated sheep red blood cells overnight at 4°C. The resultant rosettes were separated by repeat Ficoll-Hypaque density centrifugation followed by hypotonic lysis of the sheep red blood cells. The resultant T cell preparation was washed with RPMI 1640.  $1-2 \times 10^6$  T cells were incubated at 4°C for 45 min with the appropriate mAb antibody and then washed twice with RPMI 1640. A second incubation was then performed with fluoresceinated goat anti-mouse Ig (Tago Inc., Burlingame, CA), followed by two additional washes with RPMI 1640. Consent for blood drawing was obtained from all individuals.

*Neuraminidase Treatment.* Human T cells as prepared above were exposed to 10 U/ml of neuraminidase (Sigma Chemical Co., St. Louis, MO) for 45 min at 37°C. Binding to mAb 3G5 or control monoclonal A2B5 was assessed by the methods above.

S. L. Rabinowe was supported by National Institutes of Health training grant 2-T-32-AMO-7315-06, a Research and Development Award from the American Diabetes Association, and by NIH Biomedical Research Support grant RR-05673. G. S. Eisenbarth was supported by NIH grant RO1 AM-32083, and R. C. Nayak by a fellowship from the Juvenile Diabetes Foundation. Data was analyzed using CLINFO of the Brigham and Women's Hospital, Boston, MA. Address correspondence to Steven L. Rabinowe, M.D., Joslin Diabetes Center, One Joslin Place, Boston, MA 02215.

**Ganglioside Preparation and ELISA.** A Folch preparation of lipids was made from  $10^8$  human T cells (4). T cells were extracted with chloroform/methanol/water 1:2:1.4 (vol/vol) and the aqueous phase was removed and evaporated. The residue was dissolved in chloroform/methanol/water 60:30:4.5 (vol/vol), and after centrifugation the supernatant fraction was dialyzed against distilled water, lyophilized, and resuspended in methanol, aliquoted into a microtiter plate, and dried under a flow of gaseous nitrogen. Under these conditions purified gangliosides adsorb to microtiter wells. mAb 3G5 or control antibody P3X63 were then placed in each well and the microtiter plates were incubated at 4°C for 45 min. The plate was washed and peroxidase-conjugated rabbit anti-mouse Ig was incubated in each well for 30 min at room temperature. The wells were then washed and the color reaction was developed with hydrogen peroxide and *o*-phenylenediamine; the optical density was read at 492 nm.

**TLC and Direct Immunostaining of Chromatograms.** Immunostaining of TLCs was performed using an immunoperoxidase based modification of the method of Magnani (5). Briefly, a ganglioside preparation as above (from  $\sim 10^8$  sheep erythrocyte-rosetted human T cells) was divided into two lanes on an aluminum-backed high-performance TLC plate and separated by ascending chromatography in chloroform/methanol/KCl (0.25%; aqueous) 5:4:1. The plate was immersed for 90 s in a saturated solution of polyisobutyl methacrylate in hexane and then air dried. The plate was then incubated in PBS/3% BSA for 1 h. The lanes were subsequently reacted with either mAb 3G5 or control antibody P3X63 at a 1:250 dilution in PBS/3% BSA for 2 h, were given three 5-min washes in PBS/3% BSA, and were incubated for 90 min in horse radish peroxidase-conjugated rabbit anti-mouse Ig (1:100), followed by three 5-minute washes in PBS/3% BSA. Horse radish peroxidase-conjugated swine anti-rabbit Ig was then added as above. The color reaction was developed with 40 mg 4-chloro-1-naphthol in 50 ml of 50 mM Tris, pH 7.4, containing 0.012%  $H_2O_2$ .

## Results

A clear relationship between age and the percentage of circulating 3G5<sup>+</sup> T lymphocytes was found in normal human subjects ( $r = 0.85$ ,  $p < 0.001$ ; Fig. 1). The percentage of circulating T cells reacting with mAb 3G5 increased linearly from age 7 (23–30%) to age 84 (58%). In contrast, the percentage of OKT4<sup>+</sup> (Leu-3) and OKT8<sup>+</sup> (Leu-2) T cells did not vary with age (Fig. 1). The percent 3G5<sup>+</sup> T cells in pairs of monozygotic twins showed a high degree of correlation ( $r = 0.840$ ,  $p < 0.005$ ; Fig. 2). The 3G5<sup>+</sup> T cell subset was further characterized using directly fluoresceinated 3G5 and phycoerythrin-conjugated Leu-2 (suppressor/cytotoxic cells) and Leu-3 (helper cells). The majority of the 3G5<sup>+</sup> T cells were Leu-3<sup>+</sup> cells (73%) and 17% were Leu-2<sup>+</sup> cells. Of the Leu-2<sup>+</sup> circulating cells, 24% were 3G5<sup>+</sup> and of the Leu-3<sup>+</sup> cells, 45% were 3G5<sup>+</sup>. The 3G5 antibody did not bind to nonrosetting mononuclear cells as shown by immunofluorescent detection.

To determine whether 3G5 represented an activation antigen associated with aging, the percentage of cells reacting with monoclonal 3G5 were quantitated after PHA (2  $\mu$ g/ml) stimulation. In each of three experiments the percentage of 3G5<sup>+</sup> cells decreased after incubation with PHA as compared with control cultures. We found decrements of up to 90% in percentage of cells expressing the 3G5 antigen.

To determine whether the antigen on T cells is biochemically similar to that on neuroendocrine cells we treated T cells with purified neuraminidase, which removes terminal sialic acid residues of glycoproteins and gangliosides. Neura-

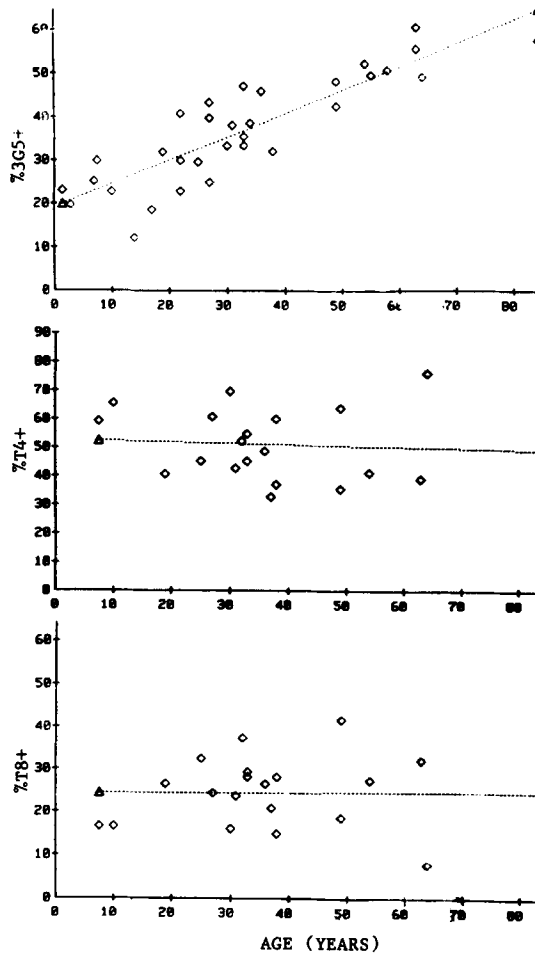


FIGURE 1. The percentage of T cells reacting with mAbs 3G5 (*top*), T4 (*middle*), and T8 (*bottom*) were analyzed on a FACS. Age (in years) is represented on the abscissa in each panel.

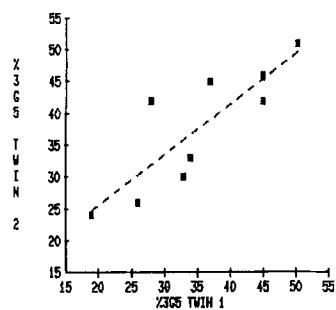


FIGURE 2. The percentage of T cells reacting with Ab 3G5 are shown for nine sets of monozygotic twin pairs. The linear regression line is shown ( $r = 0.840$ ,  $p < 0.005$ ).

minidase-treated T cells failed to react with antibody 3G5. The binding decreased to that of the nonspecific negative control (A2B5) (Fig. 3).

To further characterize the antigen, a Folch preparation of T cell gangliosides was adsorbed to microtiter wells. An ELISA using mAb 3G5 and control antibody P3X63 was prepared. The optical density was  $0.135 \pm 0.003$  for wells incubated

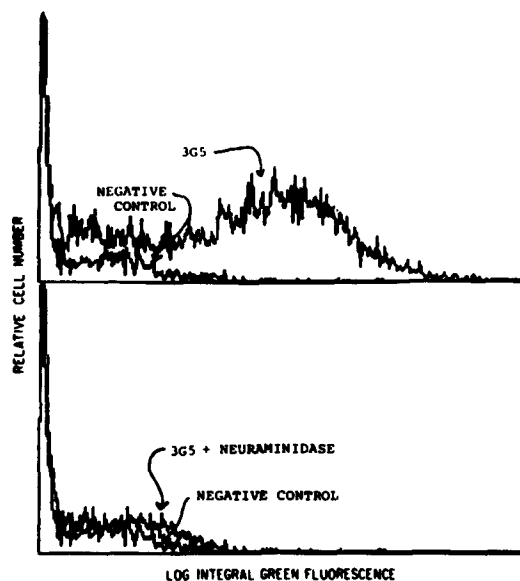


FIGURE 3. The binding of mAb 3G5 to purified human peripheral T cells (assessed by FACS analysis) is shown in the top panel (method as per Fig. 1). Nonspecific binding was assessed by a negative control (monoclonal A2B5). After exposure of T cells to neuraminidase, the binding of 3G5 is lost (bottom). Log integral green fluorescence is plotted on the abscissa. Relative cell number is plotted on the ordinate for each graph.

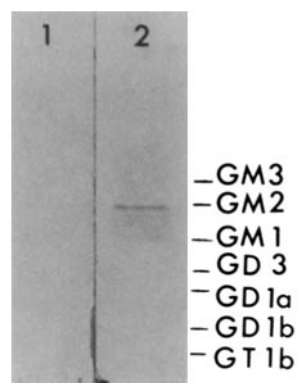


FIGURE 4. Thin layer chromatogram of a T cell ganglioside preparation stained with control mAb P3X63 (1) and 3G5 (2). The 3G5 ganglioside migrates between the GM1 and GM2 markers.

with antibody 3G5, exceeding the control ( $0.08 \pm 0.009$ ;  $p < 0.001$ ), which indicated the presence of 3G5 antigen(s) in the purified ganglioside preparation (6). We were unable to demonstrate a T cell protein reacting with antibody 3G5 on Western blots after PAGE. Using TLC the 3G5 antigen of human E-rosetted T cells migrates below GM2 and above GM1 (Fig. 4). The possibility of the detected antigen originating from the sheep erythrocytes was excluded by the detection of the same antigen in extracts of peripheral blood mononuclear cells before rosetting; furthermore, 3G5 did not bind to sheep erythrocytes, as shown by indirect immunofluorescence (data not shown).

#### Discussion

The percentage of circulating 3G5<sup>+</sup> T cells increases linearly with advancing age in normal individuals, while the percentage of OKT4 (helper-inducer) and OKT8 (suppressor/cytotoxic) cells did not vary with age. As might be expected

in individuals of the same age and phenotype, the monozygotic twin pairs showed a strong correlation for percent 3G5<sup>+</sup> T cells. 3G5<sup>+</sup> T cells are both OKT4<sup>+</sup> and OKT8<sup>+</sup> and decrease after PHA activation. Whether the increase in the proportion of T cells expressing 3G5 reflects biosynthesis of the 3G5 antigen or the uncovering of a previously cryptic antigen with age remains to be elucidated.

mAb 3G5 reacts with a ganglioside on neuroendocrine cells (2). The antigen reacting with 3G5 on the surface of T cells appeared also to be a ganglioside, in that treatment of T cells with purified neuraminidase abrogated antibody 3G5 binding, antigenic material could be extracted with chloroform/methanol and partitioned into the methanol-water phase of a Folch preparation, the mAb 3G5 reacted with a band migrating between GM1 and GM2 ganglioside markers on TLC, and we were unable to demonstrate a T cell protein reacting with antibody 3G5 on Western blots after PAGE.

Gangliosides are glycosphingolipids containing sialic acid. They have hydrophobic and hydrophilic regions and are positioned in cell membranes with the sialic acid containing carbohydrate backbone exposed to the extracellular environment. Possible roles for gangliosides include function as receptors for hormones, toxins, and viruses, cell-cell adhesion, and synaptic transmission (7–10). The remarkable diversity of cell-surface glycoconjugates and their regulation with cellular differentiation have been increasingly appreciated (11). Although once considered primarily neural antigens, gangliosides are present on thymic epithelium, red blood cells, pancreatic islets, kidney glomeruli, and malignancies, including melanoma and neuroblastoma, and as shown in this study of human T lymphocytes (12–15). The presence of gangliosides on the surface of these diverse cell types suggest a common though currently unknown function. 3G5<sup>+</sup> cells represent the first T cell subset that reflects aging in man, and we speculate that its increase may be related to the increased prevalence of autoimmunity with aging since we have observed an abnormal increase in the percentage of 3G5<sup>+</sup> T cells in patients with amidarone-induced thyroid autoimmunity and Type I diabetes (16, 17).

### Summary

A new human T cell subset defined by antineuronal ganglioside mAb 3G5 increases linearly with advancing age in man. The percentage of circulating 3G5<sup>+</sup> T cells in 21 normal individuals, quantitated by cytofluorograph analysis, increases linearly from age 7 (23–30%) to age 84 (58%) ( $r = 0.85$ ,  $p < 0.001$ ). The antigen on T cells has the biochemical properties of a ganglioside that migrates between GM1 and GM2 ganglioside markers on TLC. The 3G5 subset represents the first T cell subset that reflects aging in man.

*Received for publication 1 December 1986 and in revised form 18 February 1987.*

### References

1. Nagel, J. E., F. J. Chrest, R. S. Pyle, and W. H. Adler. 1983. Monoclonal antibody analysis, T lymphocyte subsets in young and aged adults. *Immunol. Commun.* 12:223.
2. Powers, A. C., A. Rabizadeh, R. Akesson, and G. S. Eisenbarth. 1984. Characterization

- of monoclonal antibody 3G5 and utilization of this antibody to immobilize pancreatic islet cell gangliosides in a solid phase radioassay. *Endocrinology*. 114:1.
3. Reinherz, E. L., and S. F. Schlossman. 1980. The differentiation and function of human T lymphocytes. *Cell*. 19:821.
  4. Svennerholm, L., and P. Fredman. 1980. A procedure for isolation of brain gangliosides. *Biochim. Biophys. Acta*. 617:97.
  5. Magnani, J. L., M. Brokhaus, D. F. Smith, and V. Ginsburg. 1982. Detection of glycolipid ligands by direct binding of carbohydrate binding proteins to thin layer chromatograms. *Methods Enzymol*. 83:236.
  6. Posner, M. R., D. Antoniou, J. Griffin, S. F. Schlossman, and H. J. Lazarus. 1982. An enzyme linked immunosorbent assay (ELISA) for the detection of monoclonal antibodies to cell surface antigens on viable cells. *J. Immunol. Methods*. 48:23.
  7. Weigandt, H. 1982. The gangliosides. *Adv. Neurochem*. 4:149.
  8. Fishman, P. H., and R. D. Brady. 1976. Biosynthesis and function of gangliosides. *Science (Wash. DC)*. 194:906.
  9. Valente, W. A., P. Vitti, Z. Yavin, C. M. Rotella, E. F. Grollman, R. S. Torcafondi, and L. D. Kohn. 1982. Monoclonal antibodies to the thyrotropin receptor: stimulating and blocking antibodies derived from the lymphocytes of patients with Graves disease. *Proc. Natl. Acad. Sci. USA*. 79:6680.
  10. Markwell, M. A., L. Svennerholm, and J. C. Paulson. 1981. Specific gangliosides function as host cell receptors for Sendai virus. *Proc. Natl. Acad. Sci. USA*. 78:5406.
  11. Feizi, T. 1985. Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. *Nature (Lond.)*. 314:53.
  12. Eisenbarth, G. S., K. Shimizu, M. A. Bowring, and S. Wells. 1982. Expression of receptors for tetanus toxin and monoclonal A2B5 by pancreatic islet cells. *Proc. Natl. Acad. Sci. USA*. 79:5066.
  13. Haynes, B. F., K. Shimizu, and G. S. Eisenbarth. 1983. Identification of human and rodent thymic epithelium using tetanus toxin and monoclonal antibody A2B5. *J. Clin. Invest*. 71:9.
  14. Pukel, C. S., K. O. Lloyd, L. R. Travassos, W. G. Dippold, H. F. Oettgen, and L. J. Old. 1982. GD3, a prominent ganglioside of human melanoma. Detection and characterization by mouse monoclonal antibody. *J. Exp. Med*. 155:1133.
  15. Kemshead, J. T., F. S. Walsh, J. Pritchard, and M. Greaves. 1981. Monoclonal antibody to ganglioside GQ discriminates between hematopoietic stem cells and infiltrating neuroblastoma tumor cells in bone marrow. *Int. J. Cancer*. 27:447.
  16. Rabinowe, S. L., P. R. Larsen, E. M. Antman, K. L. George, P. L. Friedman, R. A. Jackson, and G. S. Eisenbarth. 1986. Autoimmune thyroid disease: evaluation of a new monoclonal antibody defined T cell subset. *Am. J. Med*. 81:53.
  17. Rabinowe, S. L., K. George, and R. Jackson. 1985. Lymphocyte abnormalities in individuals prior to overt diabetes mellitus or at risk for Type I diabetes mellitus. *Diabetes*. 34:268. (Abstr.)