ETHNIC DIFFERENCES IN THE LYMPHOCYTE PROLIFERATIVE RESPONSE INDUCED BY A MURINE IgG1 ANTIBODY, Leu-4, TO THE T3 MOLECULE

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Closely linked to the human T cell receptor for antigen is the T3 molecule identified by the murine monoclonal antibody OKT3 (1, 2). This IgG2a antibody consistently induces polyclonal proliferation of T cells (3). In contrast, an IgG1 antibody to the T3 molecule, Leu-4, is mitogenic for cells from some, but not all, individuals (4–6). Leu 4- and OKT3-induced mitogenic responses depend upon an accessory function of monocytes or macrophages, and circumstantial evidence suggests that genetic variations in the Fc-γ receptor of macrophages determines responsiveness or nonresponsiveness to the γ_1 Leu-4 antibody (4–6).

We have extended the analysis of the mitogenic effects of isotypically diverse antibodies to the T3 molecule, and have used this assay to examine genetically diverse population groups. The results reveal striking east-west ethnic differences in Leu-4 responsiveness and implicate the Fc-γ receptor on monocytes as the important variable.

Materials and Methods

Blood Donors and Cell Preparations. Blood samples were collected from healthy individuals belonging to six ethnic groups; a total of 141 donors included 40 Caucasians, 27 Negroes, 11 Indians, 27 Japanese, 24 Chinese, and 12 American Indians. Their ethnic origin was determined by oral declaration; there was no known racial mixture in the ancestors of the donors. All of the donors tested were permanent or temporary residents in the United States, except for 18 donors from Japan. The American Indian donors live in New Mexico and belong to six different tribes, Navajo ($n = 3$), Pueblo ($n = 3$), Laguna ($n = 3$), Acoma ($n = 1$), Hopi ($n = 1$), and Sioux ($n = 1$). Negro donors included 17 Americans and 10 African students from Nigeria ($n = 6$), Uganda ($n = 2$), and Ethiopia ($n = 2$).

Mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation (7). Monocyte/macrophages were purified by plastic adherence while lymphocytes were purified by a two-step technique with initial depletion of adherent cells on a plastic surface and then by passage through a nylon wool column (7).

Monoclonal Antibodies. The monoclonal antibodies OKT3 (IgG2a), Leu-4 (IgG1), and

TABLE I

A Comparison of the Mitogenic Capabilities for the Monoclonal Antibodies OKT3, Leu-4, and 38.1

Donor		Sex	Age	Monoclonal antibodies (isotype)			
				None	OKT3 (IgG2a)	Leu-4 (IgG1)	38.1 (IgM)
Caucasian	1	M	27	1,490 ± 145	19,531 ± 1,520	19,154 ± 1,397	463 ± 21
	2	M	28	315 ± 126	40,047 ± 5,849	33,093 ± 2,319	225 ± 46
	3	M	31	979 ± 39	34,268 ± 4,589	49,286 ± 11,352	1,181 ± 534
	4	F	29	426 ± 50	45,492 ± 3,479	3,165 ± 110	411 ± 108
	5	F	30	280 ± 39	26,200 ± 2,086	422 ± 59	272 ± 36
Negro	1	M	40	542 ± 123	30,793 ± 2,408	27,448 ± 3,492	562 ± 48
	2	M	45	651 ± 176	25,670 ± 1,307	458 ± 48	456 ± 52
	3	F	22	641 ± 65	13,338 ± 542	32,835 ± 2,440	893 ± 688
	4	F	31	356 ± 89	14,303 ± 1,300	16,437 ± 768	470 ± 128
	5	F	42	308 ± 66	25,821 ± 1,604	24,452 ± 1,669	442 ± 22
Indian	1	M	29	416 ± 46	26,744 ± 1,427	355 ± 22	389 ± 48
	2	M	30	490 ± 113	13,200 ± 1,215	15,717 ± 2,345	515 ± 70
	3	M	49	383 ± 15	24,016 ± 2,417	3,234 ± 421	622 ± 82
	4	F	31	298 ± 95	11,444 ± 1,020	244 ± 21	286 ± 22
	5	F	40	379 ± 76	21,330 ± 795	7,418 ± 672	547 ± 89
Japanese	1	M	31	361 ± 105	31,251 ± 1,929	519 ± 161	572 ± 24
	2	M	32	652 ± 377	39,012 ± 6,358	361 ± 36	522 ± 74
	3	M	42	213 ± 64	23,129 ± 2,910	583 ± 14	200 ± 95
	4	F	29	211 ± 78	42,549 ± 116	189 ± 35	451 ± 162
	5	F	57	166 ± 64	11,567 ± 1,496	172 ± 25	268 ± 36

38.1 (IgM), all of which react with the T3 molecule, were used at a concentration of 100 μg/ml unless otherwise stated (8–10). For cell cultures and immunofluorescence tests, 10^6 mononuclear cells were treated with 10 μl of each antibody at 4°C for 30 min. After incubation the cells were washed twice with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 50 μg/ml gentamycin to eliminate excess antibody. These antibody-treated cells were developed with fluorescein-conjugated goat antibodies to mouse Ig for analysis of their staining intensities with a fluorescence-activated cell sorter (FACS-IV; B-D FACS Systems, Sunnyvale, CA) (7).

Cell Cultures.   Cells were cultured in plastic, 96-well, flat-bottomed culture plates (Costar, Cambridge, MA) at 37°C in a humidified atmosphere containing 5% CO_2 in air (7). 200 μl of pretreated cells suspended at a concentration of 1 × 10^6/ml in medium were cultured in triplicate wells for 3 d. To measure [^{3}H]thymidine incorporation, the cells were pulsed with 0.5 μCi/well [^{3}H]thymidine 8 h before the cultures were terminated.

Results

In an initial experiment we measured the proliferative responses of blood mononuclear cells (MNC)[1] from 20 healthy donors belonging to four ethnic groups (Caucasian, Negro, Indian, and Japanese) after pretreatment of the cells with three monoclonal anti-T3 antibodies of different isotypes (Table I). Regardless of sex, age, and race of the donor, the murine IgG2a monoclonal antibody OKT3 induced proliferation of MNC from all individuals tested, whereas the IgM monoclonal antibody 38.1 was not mitogenic for cells from any of these donors (even at doses up to 1 mg/ml of antibody). In contrast, the results with the IgG1 monoclonal Leu-4 antibody were heterogeneous. MNC from some donors proliferated in response to Leu-4 at levels that were comparable to the OKT3 mitogenic effect (e.g., Caucasians 1–3), whereas MNC from other donors exhibited a lower proliferative response to Leu-4 than to the antibody OKT3

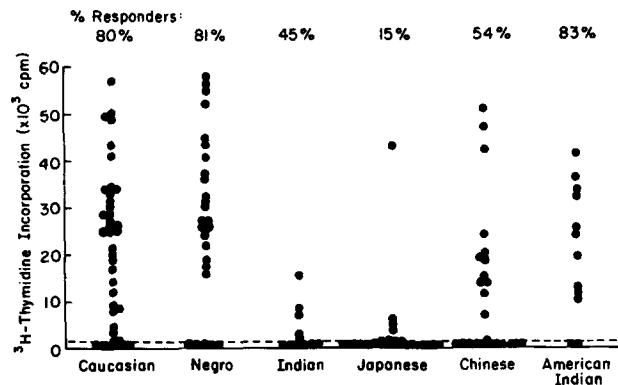[1] *Abbreviations used in this paper:* MNC, mononuclear cells.

FIGURE 1. Racial differences in the mitogenic effect of the Leu-4 (IgG1) antibody. Blood mononuclear cells were cultured for 3 d after pretreatment with the Leu-4 antibody. The actual [^{3}H]thymidine incorporation measurements (cpm) were plotted for the donors of different races. A stimulation index of >3 (dotted line) was considered a positive response.

(e.g., Caucasian 4 and Indian 3). The remaining donors lacked MNC responsiveness to Leu-4 stimulation; e.g., all five Japanese donors were nonresponders. Repetition of the assay (at least five times) in several individuals revealed that the level of responsiveness was stable over a period of >6 mo. These results suggested important racial differences in the Leu-4-induced proliferative response.

We then examined Leu-4 responses in a series of individuals from six ethnic groups. The majority of Caucasians and Negroes (80 and 81%, respectively) showed MNC responsiveness to Leu-4 stimulation (Fig. 1). In contrast, many Indians, Japanese, and Chinese were nonresponders. Among the Indians who exhibited Leu-4 responsiveness, the magnitude of cell proliferation was relatively low (<20,000 cpm). There was no difference in the proportion of Leu-4 responders between the 17 American Negroes (82% Leu-4 responders) and the 10 African Negroes (80%), nor between the 9 Japanese living in the United States and the 18 Japanese living in Japan. There was a higher proportion of Leu-4 responders among Chinese than among Indian and Japanese donors. To our surprise, American Indians exhibited the same incidence of Leu-4 responsiveness (83%) as Caucasians (80%) and Negroes (81%). Because of the great variability in the magnitude of the MNC responses to Leu-4 within ethnic groups, a nonparametric analysis of variance was performed on the ranks of the responses (Duncan's multiple range test). A significant difference was demonstrated between the donor groups of Negroes, American Indians, and Caucasians compared with the Chinese, Indians, and Japanese ($P < 0.0001$). There was no clear sex difference in Leu-4 responsiveness. Thus, the frequencies of Leu-4 responders among men and women were 72 vs. 86% in Caucasians ($n = 18$ and 22), 79 vs. 88% in Negroes ($n = 19$ and 8), 43 vs. 50% in Indians ($n = 7$ and 4), 14 vs. 15% in Japanese ($n = 14$ and 13), 67 vs. 47% in Chinese ($n = 8$ and 15), and 88 vs. 75% in American Indians ($n = 8$ and 4).

The monoclonal antibodies OKT3, Leu-4, and 38.1 all identify the same structure of ~22 kilodalton (kD) and 28 kD on T cells (8–10). Despite their
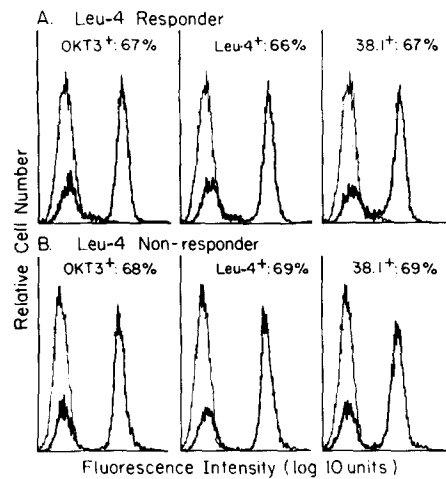
FIGURE 2.   Lymphocyte binding efficiencies of OKT3 (IgG2a), Leu-4 (IgG1), and 38.1 (IgM) monoclonal antibodies. Mononuclear cells from a Leu-4 responder and a nonresponder were incubated with one of the monoclonal antibodies and developed with fluorescein-conjugated goat antibodies to mouse Ig. The first peak in each figure is the background fluorescent reactivity with the developing antibody only.

distinctive mitogenic capabilities, these antibodies appeared to bind equally well to blood mononuclear cells; when analyzed by indirect immunofluorescence, there were no noticeable differences in either the staining intensity or the proportion of stained cells (Fig. 2).

Proliferative responses were examined as a function of concentration (0.01–20 μg/ml) of the OKT3 and Leu-4 antibodies using MNC from two Leu-4 responders and two nonresponders. The OKT3 dose-response curves were similar for MNC cultures from all four donors; no response was observed at 0.1–0.2 μg/ml and maximal responses were observed at concentrations of 1–2 μg/ml. Similar Leu-4 dose-response curves were observed for the MNC from the two Leu-4 responders, while MNC from the other two donors were unresponsive over the entire range of Leu-4 concentrations.

Finally, we examined whether accessory monocytes were contributing to the differential effects of the T3 antibodies. When purified lymphocytes were cultured without monocytes, there was no proliferation after pretreatment with OKT3, Leu-4, or 38.1, even after 2 wk of culture. Purified granulocytes exhibited no accessory function for lymphocytes treated with any of these antibodies. The addition of monocytes restored lymphocyte proliferation in cultures of OKT3-treated lymphocytes, but not in cultures with 38.1-treated lymphocytes. Similar results were obtained for both autologous and allogeneic combinations of lymphocytes and monocytes. In the case of Leu-4-treated lymphocytes, however, only monocytes from Leu-4 responder donors could restore the proliferation of lymphocytes from either Leu-4 responder or Leu-4 nonresponder donors (Fig. 3). A relatively high number of monocytes from Leu-4 low responders was required for a maximal proliferative response, whereas even large numbers of monocytes (up to 40%) from Leu-4 nonresponders could not provide an accessory function in the Leu-4 response. On the other hand,
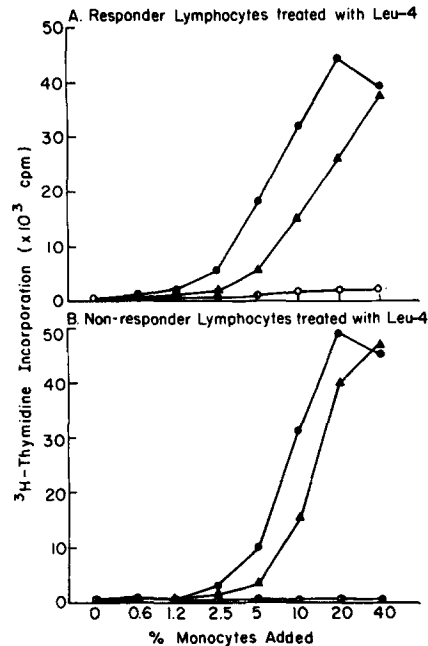
FIGURE 3. Monocytes from Leu-4 responders, but not from Leu-4 nonresponders, perform an accessory function in the proliferative response of Leu-4 antibody-treated lymphocytes. Graded numbers of monocytes were added to 2×10^5 lymphocytes/well. Representative experiments using lymphocytes from a Leu-4 responder (A) and a Leu-4 nonresponder (B) are shown using combinations of allogeneic monocytes from a high Leu-4 responder (●), a low Leu-4 responder (▲), and a Leu-4 nonresponder (○). The [^{3}H]thymidine incorporation in control cultures of untreated lymphocytes and graded numbers of allogeneic monocytes was always <3,000 cpm on day 3, the assay day in these experiments.

monocytes from Leu-4 nonresponders were found to be normally capable of providing accessory function in the proliferative responses of T cells to phytohemagglutinin and concanavalin A and of T and B cells to pokeweed mitogen (data not shown).

Discussion

The present studies reveal interesting ethnic differences in accessory monocyte function for the γ_1 Leu-4-induced proliferative response of T cells. A high proportion of Asians, including Indian, Japanese, and Chinese, were either nonresponders or low responders in this assay. In contrast, a high proportion of both Caucasians and Negroes were responders. Although a significant portion of people in India have both Asian and Indo-European ancestry (a common ancestry of Caucasoids), the monocyte accessory function of Indian individuals was clearly distinguishable from that of Caucasians. Another unexpected result was the high proportion of Leu-4 responders among North American Indians, whose ancestors are thought to have migrated from Asia (Siberia).

Two possible explanations for the differences in the Leu-4 responder rates for American Indians and the Asian populations studied here are as follows. One is the effect of natural selection after migration to the North American continent.

For example, a major epidemic caused by a "new" infectious disease could have selected for responders at the expense of nonresponders, as has been proposed to explain the high degree of HLA homozygosity in American Indians (11). An alternate hypothesis may be suggested from our data. Since some individuals of Asian origin, especially Chinese, are good responders in the Leu-4 assay, it is possible that the ancesters of American Indians belonged to a Mongolian group of high Leu-4 responders.

Although MNC responsiveness to Leu-4 is a heritable trait, family studies suggest that this response is not regulated by a single dominant gene of HLA-A, B, C, or D (5). The present analysis and other studies (5, 6) suggest that Leu-4 responsiveness is unrelated to either sex or age. This study has also shown that the different mitogenic effects of the three monoclonal antibodies with distinct isotypes, OKT3 (IgG2a), Leu-4 (IgG1), and 38.1 (IgM), are not due to differential reactivity with T lymphocytes but rather may be determined by monocyte interactions with the Fc domains of lymphocyte-bound anti-T3 antibodies. The requirement of the Fc portion of anti-T3 antibodies for induction of a proliferative response was suggested in a previous study (5) in which Fab fragments were found to be nonmitogenic. Fc-γ receptors on human monocytes appear to have graded affinities for murine immunoglobulin molecules of different isotypes; these receptors bind strongly to murine immunoglobulin of the IgG2a isotype but weakly to other Ig isotypes (12). The heterogeneity of the Leu-4 (IgG1) response thus suggests genetic polymorphism in the γ_1 affinity of Fc receptors on monocytes among the different ethnic groups.

Different ethnic groups may vary in their susceptibility to certain diseases, including infections, autoimmune diseases, and malignancies (13, 14). Genetic polymorphism in accessory function of monocytes, as noted here, might reflect one basis for variable immune responses and associated disease. Further population studies of Leu-4-induced MNC responsiveness may also help to elucidate ethnic origins and population migration patterns.

Summary

The mitogenic effects of isotypically diverse antibodies to the T3 molecule were examined in genetically diverse population groups. Whereas the OKT3 antibody (IgG2a) was mitogenic for blood mononuclear cells from all individuals tested, the 38.1 antibody (IgM) was consistently nonmitogenic. In contrast, studies of the mitogenic effects of the Leu-4 antibody (IgG1) revealed striking ethnic differences. More than 80% of Caucasians and Negroes were good Leu-4 responders, whereas most individuals of Asian origin, including Indian, Japanese, and Chinese, were either Leu-4 nonresponders or Leu-4 low responders. However, the majority of American Indians, as well as a significant minority of Chinese, were good responders. Cell separation studies confirmed that monocytes govern the different mitogenic effects of the anti-T3 antibodies. The results reveal interesting ethnic differences in monocyte accessory function probably mediated via the Fc-γ receptor, in the stimulation of T lymphocytes by an IgG1 antibody against the T3 molecule.

References

1. Chang, T. W., P. C. Kung, S. P. Gingras, and G. Goldstein. 1981. Does OKT3 monoclonal antibody react with an antigen-recognition structure on human T cells? *Proc. Natl. Acad. Sci. USA.* 78:1805.

2. Reinherz, E. L., S. Meuer, K. A. Fitzgerald, R. E. Hussey, H. Levine, and S. F. Schlossman. 1982. Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell.* 30:735.

3. Van Waume, J. P., J. R. De Mey, and J. G. Goossens. 1980. OKT3: a monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. *J. Immunol.* 124:2708.

4. Van Waume, J. P., and J. G. Goossens. The mitogenic activity of OKT3 and anti-Leu-4 monoclonal antibodies: a comparative study. *Cell Immunol.* 77:23.

5. Kaneoka, H., G. Perez-Rojas, T. Sasasuki, C. J. Benike, and E. G. Engleman. 1983. Human T lymphocyte proliferation induced by a pan-T monoclonal antibody (anti-Leu4): heterogeneity of response is a function of monocytes. *J. Immunol.* 131:158.

6. Tax, W. J. M., H. W. Willems, P. P. M. Reekers, P. J. A. Capel, and R. A. P. Koene. 1983. Polymorphism in mitogenic effect of IgG1 monoclonal antibodies against T3 antigen on human T cells. *Nature (Lond.).* 304:445.

7. Abo, T., and C. M. Balch. 1982. Characterization of HNK-1$^+$ (Leu7) human lymphocytes II. Distinguishing phenotypic and functional properties of natural killer cells from activated NK-like cells. *J. Immunol.* 129:1758.

8. Borst, J., M. A. Prendiville, and C. Terhorst. 1983. The T3 complex on human thymus-derived lymphocytes contains two different subunits of 20 kDa. *Eur. J. Immunol.* 13:576.

9. Rinnooy Kan, E. A., C. Y. Wang, L. C. Wang, and R. L. Evans. 1983. Noncovalently bonded subunits of 22 and 28 kd are rapidly internalized by T cells reacted with anti-Leu4 antibody. *J. Immunol.* 131:536.

10. Hasen, J. A., and P. J. Martin. 1982. Human T cell differentiation antigens. *In* Hybridoma in Cancer Diagnosis and Treatment. M. S. Mitchell and H. F. Dettgen, editors. Raven Press, New York. 75–88.

11. Bodmer, W. F., H. Cann, and A. Piazza. 1972. Differential genetic variability among polymorphisms as an indicator of natural selection. *In* Histocompatibility Testing. J. Dausset and J. Colombani, editors. Munksgaard, Copenhagen. 753–767.

12. Steplewski, Z, M. D. Lubeck, and H. Koprowski. 1983. Human macrophages armed with murine immunoglobulin G$_{2a}$ antibodies to tumors destroy human cancer cells. *Science (Wash. DC).* 221:865.

13. Kolonel, L. N. 1980. Cancer patterns of four ethnic groups in Hawaii. *J. Natl. Cancer Inst.* 65:1127.

14. Miller, R. W., P. A. Gilman, and H. Sugano. 1981. Differences in lymphocytic disease in the United States and Japan. *J. Natl. Cancer Inst.* 67:739.