Brief Definitive Report

ANTICLONOTYPIC MONOCLONAL ANTIBODIES INDUCE PROLIFERATION OF CLONOTYPE-POSITIVE T CELLS IN PERIPHERAL BLOOD HUMAN T LYMPHOCYTES

Evidence for a Phenotypic (T4/T8) Heterogeneity of the Clonotype-positive Proliferating Cells

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Monoclonal antibodies (mAb) directed to the molecular complex (formed by T3 molecules and by molecules carrying clonotypic determinants) serving as receptor for antigen on human T cells can induce cell activation, possibly leading to interleukin 2 (IL-2) release and cell proliferation (1, 2). However, although antibodies directed to the monomorphic T3 molecules induce polyclonal T cell responses, antibodies specific for the clonotypically restricted structures (termed Ti) only trigger cells bearing the relevant clonotype (3). As a consequence, the functional effect(s) of anti-Ti mAb have been studied exclusively in cells belonging to the corresponding T cell clones (used for immunization). It is conceivable, however, that resting peripheral T cells carrying a given clonotype may be susceptible to triggering by the corresponding anticlonotypic antibodies and undergo proliferation. In this report we show that three mAb (designated JTi_{1-3}), originally raised against the clonotypic structure of a cloned variant of the IL-2-producing Jurkat leukemia cell line (designated JA3) (4, 5), and cross-reacting with 0.5–1% of peripheral blood (PB) lymphocytes, promote (late) proliferation of PB T lymphocytes. This proliferation mostly reflects the expansion of clonotype-positive (JTi^+) T cells that express either the $T4^+$ or the $T8^+$ phenotype. The antibody-stimulated JTi^+ PB populations express disulphide-linked heterodimeric surface molecules similar to those of JA3 cells.

Materials and Methods

Cells. Mononuclear cells were isolated from PB and, when necessary, adherent cells were removed by incubation on plastic petri dishes (6). Purified T lymphocytes were isolated by rosetting PB lymphocytes with sheep erythrocytes as previously described (11). Mixed lymphocyte culture (MLC)- or phytohemagglutinin (PHA)-activated T cells were obtained as previously described (5, 6) and further cultured in IL-2-containing supernatants for various periods of time before fluorescence-activated cell sorter (FACS) analysis. The 6-thioguanine-resistant JA3 cloned cell line was selected, as previously described (4).

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mAb, Immunofluorescence Staining, and FACS Analysis. JTi_{1-3} mAb were obtained by immunizing mice with cells from a cloned variant of the Jurkat leukemic T cell line (termed JA3) (surface phenotype: $T11^+$, $T3^+$, $3A1^+$, $T6^-$, $T4^-$, $T8^-$, HLA-DR^-, Tac^-, $4F2^+$) (4). They were shown to recognize clonotypically restricted, heterodimeric structures of JA3 cells (4). JA3 produced large amounts of IL-2 upon stimulation with PHA (anti-T3 or anti-JTi antibodies) in conjunction with phorbol myristate acetate (PMA) or adherent cells (5). The UCHT-1 (anti-T3), B9-4 (anti-T8), D1-12 (anti-HLA-DR), anti-Tac, and the 4F2 mAb were generous gifts of Drs. Crumpton, Malissen, Accolla, Waldmann, and Fauci (5). The OKT4A mAb was purchased from Ortho Pharmaceutical Corp., (Raritan, NJ). The anti-Leu-1 mAb was purchased from Becton-Dickinson, Basel, Switzerland. The techniques used for staining cells by immunofluorescence, for FACS analysis and for cell sorting, have been described in detail (6).

Characterization of Radioiodinated Cell Surface Proteins. $\sim 10^7$ JA3 cells or anti-JTi_2–stimulated lymphocytes (7 d plus 3 d in Ab-free media) were surface labeled with ^{125}I using lactoperoxidase-glucose-oxidase–catalyzed iodination, and were lysed as described (7). The material immunoprecipitated by anti-JTi_2 mAb was analyzed on 11% (reduced) or 8% (nonreduced) discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide gels as described (4, 7).

Stimulation of PB Lymphocytes with mAb. Purified T lymphocytes (or unfractionated PB mononuclear cells) were cultured in round-bottomed microwells (10^5 cells per well) with one or another mAb in RPMI 1640 culture medium supplemented with 10% fetal calf serum (5). Anti-JTi mAb were used at a 1:100 final dilution of the hybridoma culture supernatant (or, in some experiments, at a 1:5000 dilution of the ascitic fluid). UCHT-1 was used at a 1:5000 dilution of the ascitic fluid, whereas the anti-Leu-1 mAb was used at a 1:500 dilution of the stock solution. Cultures were continued for different intervals, and 0.5 μCi of tritiated thymidine ([^{3}H]TdR) was added 18 h before collection and measurement of TdR uptake (5).

Results and Discussion

As previously shown (4, 5), anti-JTi_{1-3} antibodies react strongly with JA3 cells but they do not appear to bind to PB resting or activated T cells as assessed by indirect immunofluorescence and FACS analysis. However, by fluorescence microscope examination, 0.5–1% of cells were found to be JTi^+ in three different individuals. When PB lymphocytes were cultured in the presence of any one of the anti-JTi mAb, a detectable proliferation started at day 5 and reached maximal levels at day 7–9 (Fig. 1). No proliferation was detected in control cultures

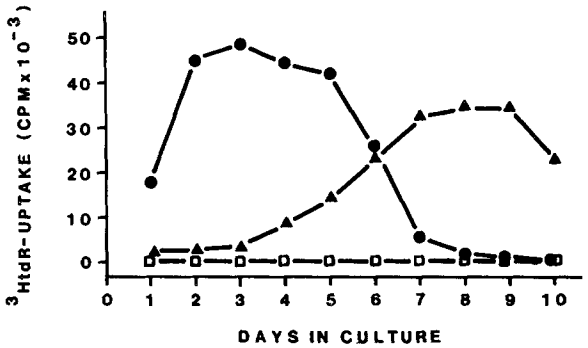

FIGURE 1. Anticlonotypic antibodies induce (late) proliferative responses of PB human mononuclear cell populations. Ficoll/Hypaque-purified mononuclear cells were cultured in round-bottomed microwells (10^5 cells per well) with either one of the following mAb: anti-JTi_2 (▲), anti-Leu-1 (□), UCHT1 (●). Cultures were continued for various periods of time before scoring for [^{3}H]TdR uptake.

containing other mAb of the same IgG2A subclass, such as 4F2 (not shown) or Leu-1 (Fig. 1). Similar results were obtained in 20 different individuals: in all instances a measurable cell proliferation began at day 5–6 and peaked at day 7–9. Polyclonal stimulation of the same cell populations with anti-T3 antibody resulted in a strong proliferative response as early as at day 2–3 of culture. Removal of adherent cells abrogated proliferative responses of PB lymphocytes to both anti-JTi and anti-T3 mAb (data not shown). To assess whether cell proliferation reflected, at least in part, the clonal expansion of cells carrying the JTi clonotype (or crossreacting clonotypes), lymphocytes cultured for 7 d with anti-JTi_2 mAb were harvested, washed extensively, and further kept in antibody-free medium for 48 h before staining with anti-JTi or anti-T3 mAb and analysis by FACS. The rationale for this experimental procedure was that antibodies directed to the T3/Ti receptor complex are known to induce a reversible loss of these antigens from the cell surface ("modulation") (1). >90% of the large (blast) cells were found to be $T3^+$; more importantly, 70–90% expressed JTi determinants (see Fig. 2). Time course experiments showed that, while virtually no JTi^+ cells were detectable at day 0, few positive cells were present after a total culture period of 4 d and that they represented ~20% of the cells at day 6. At day 10, as much as 40% of the total cells recovered were JTi^+ (not shown). Moreover, similar proportions of JTi^+ cells, after stimulation with anti-JTi_2 mAb, could be detected with any of the anti-JTi mAb, including the anti-JTi_1 mAb that recognizes on the JA3 T cell receptor an epitope distinct from that recognized by anti-JTi_2 or anti-JTi_3 mAb (4) (not shown). On the other hand, in control experiments in which cells were stimulated with anti-T3 mAb or cultured in the presence of PHA or allogeneic cells, <1% JTi^+ cells were detectable after 4–15 d of culture. The increase in the proportion of JTi^+ cells was paralleled by the expression of T cell activation markers such as HLA-DR, the receptor for IL-2 and 4F2 antigens. Thus, for example, after 8 d of culture, 38% of the cells were stained by anti-JTi_2 mAb, whereas 38, 42, and 45% were $HLA\text{-}DR^+$, IL-2 receptor–positive, or $4F2^+$, respectively, as assessed by specific mAb and FACS analysis. In addition, these antigens appeared to be expressed by the same large cells that expressed JTi determinants. In some experiments, JTi^+ blasts were further purified by FACS and cultured for an additional 3 d in IL-2-containing supernatant (6). Cells were then analyzed for expression of JTi determinants and also for T4 and T8 subset-specific surface antigens. Virtually 100% of the sorted cells expressed T3 and JTi determinants (Fig. 2, *D* and *E*) whereas approximately 55 and 40% were $T4^+$ and $T8^+$, respectively; >90% of cells were positive when stained simultaneously with anti-T8 and anti-T4 mAb, indicating that the corresponding antigens were expressed by different cells (Fig. 2*F*).

Next, we compared the anti-JTi–reactive molecules in T cell populations stimulated for 7 d with anti-JTi_2 mAb (and cultured for 72 h in antibody-free media) with those expressed on JA3 leukemia cells. Cells were surface labeled with ^{125}I by the lactoperoxidase-glucose-oxidase–catalyzed iodination method (7). They were then lysed and the resulting cell extracts immunoprecipitated with anti-JTi_2. Upon SDS–polyacrylamide gel electrophoresis (PAGE), the molecules recognized by anti-JTi_2 in both lysates displayed an M_r of ~85,000 in nonreducing conditions. Under reducing conditions, the M_r 85,000 band was

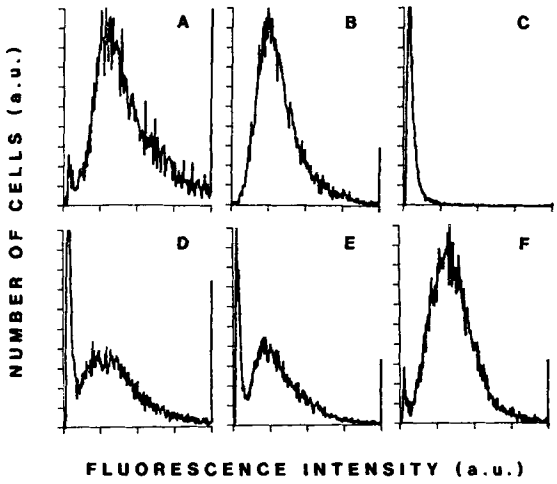

FIGURE 2. Phenotypic heterogeneity of PB-derived JTi$^+$ cells. JTi$^+$ cells were isolated by FACS sorting from PB mononuclear cells that had been cultured for 12 d with anti-JTi$_2$ mAb. After sorting and extensive washing, cells were cultured for 3 d in antibody-free media containing exogenous IL-2 and then stained with one or another of the following mAb: anti-T3 (*A*), anti-JTi$_2$ (*B*), anti-T4 (*D*), anti-T8 (*E*). In *C* control cells were stained with the second reagent only. (*F*) Cells were stained simultaneously with anti-T4 and anti-T8 mAb. Positive cells were >90%, indicating that T4 and T8 antigens were expressed by different cells.

resolved in bands of approximately 40,000, 45,000, and 49,000 (Fig. 3). The presence of a third band in anti-JTi immunoprecipitates from Jurkat-derived clones is in line with previous data from our own and others' laboratories (4, 5). We therefore conclude that molecules carrying clonotypic determinants in JA3 cells and in lymphocyte populations stimulated with anti-JTi antibodies are similar.

Taken together, the present results indicate that antibodies directed to clonotypic structures (or recognizing framework determinants present on small subsets of T cell receptors) can induce extensive proliferation of the few clonotype-positive T cells present in normal T cell populations. Similar data have been recently reported by Bigler et al. (8) using the S511 mAb, which recognizes the human T cell antigen receptor expressed by a T cell leukemia and by 1–2% of peripheral T cell. Whether any anticlonotypic antibody may behave in a similar fashion remains to be determined. It is likely, however, that antibody-induced clonal proliferation of lymphocytes expressing a given clonotype may greatly depend on the frequency of their precursors in the original T cell population, as well as on the presence of IL-2-producing cells among such Ti$^+$ precursors. It is also likely that the stimulatory capability of anticlonotypic mAb is contingent on the affinity and class (or the subclass) of the antibody itself and on the epitope recognized; it should be noted, however, that JTi$_1$ and JTi$_2$ have been shown to recognize different epitopes of the JA3 T cell receptor (4). Our results also imply that (at least some) anticlonotypic antibodies can replace antigen, as a triggering signal, in normal resting T cells. This observation appears of interest since, up to now, anti-Ti antibodies have been shown to mimic the effect of specific antigen only at the level of the corresponding Ti$^+$ T cell clones, which are represented always by activated cells (3, 4). Another remarkable observation is that a given

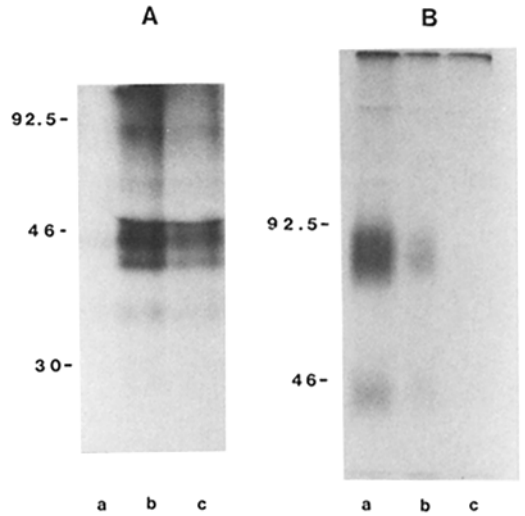

FIGURE 3. Clonotype-specific anti-JTi mAb immunoprecipitate similar molecules from JA3 cells or from PB lymphocyte populations stimulated with anti-JTi antibody. (*A*) Analysis of anti-JTi–reactive molecules by SDS-PAGE under reducing conditions. (Lane *a*) OKT6 mAb nonreactive with activated mature T cells; (lane *b*) anti-JTi_2 mAb–reactive molecules precipitated from JA3; (lane *c*) anti-JTi_2 mAb–reactive molecules precipitated from anti-JTi–stimulated PB lymphocytes. (*B*) Analysis of anti-JTi–reactive molecules by SDS-PAGE under nonreducing conditions. (Lane *a*) Anti-JTi_2 mAb–reactive molecules precipitated from JA3; (lane *b*) anti-JTi_2 mAb–reactive molecules precipitated from anti-JTi–stimulated PBL; (lane *c*) OKT6 mAb nonreactive with activated mature T cells.

anticlonotypic mAb can activate both $T8^+$ and $T4^+$ JTi^+ precursor cells, indicating that T cells belonging to different subsets can use the same clonotypic structure. It remains to be determined how this finding relates to the fact that $T4^+$ cells recognize antigen in association with major histocompatibility complex (MHC) class II molecules, whereas $T8^+$ cells recognize antigens in association with MHC class I molecules (9).

Summary

Three previously selected monoclonal antibodies (mAb) directed against the clonotypic structure of a variant (termed JA3) of the interleukin 2 (IL-2)-producing Jurkat leukemia cell line (anti-JTi_{1-3} mAb) were found to induce an adherent cell–dependent proliferation of peripheral blood T cells in 20 different donors. Unlike the early cell proliferation induced by anti-T3 mAb, anti-JTi mAb–induced proliferation was detectable at day 5–6 of culture and reached peak levels at day 7–9. Less than 1% JTi^+ cells were consistently detected in the starting peripheral blood lymphocytes or in control cultures in which cells were stimulated with anti-T3, phytohemagglutinin, or allogeneic cells. However, JTi^+ cells were found in increasing proportions after culture with anti-JTi mAb and they were mostly represented by large blast cells expressing either the T4 or the T8 antigen, together with typical activation antigens including HLA-DR, IL-2 receptor, and 4F2. Immunoprecipitation experiments and sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed that anti-JTi–reactive molecules

present on antibody-stimulated lymphocytes or on JA3 cells were similar, disulphide-linked heterodimeric structures.

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