Brief Definitive Report

A NOVEL SUBSET OF HUMAN LYMPHOCYTES WITH A T CELL RECEPTOR-γ COMPLEX

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We have recently described a series of human fetal cloned cell lines that express CD3 proteins but not the α/β T cell receptor (TCR) (1). One of these clones, termed F6C7, was used for mice immunizations. An mAb, designated anti-NKFi was found to identify on F6C7 lymphocytes a CD3-associated γ chain (2, 3) expressed either as a homodimer or possibly as part of an heterodimer (note that a δ chain has remained undetected on F6C7 cells). Anti-NKFi was shown to have a highly restricted clonotypic reactivity and could not therefore be used for characterization of lymphocyte subpopulations with a TCR-γ complex. Here, we describe a novel mAb, anti-Ti-γA, which immunoprecipitates the same γ protein as anti-NKFi. However, the Ti-γA determinant corresponds to a public epitope of this molecule, the expression of which delineates a unique subset accounting for ~3% of human circulating lymphocytes.

Materials and Methods

mAbs and Immunofluorescence Analyses. Anti-Ti-γA was produced in mice after immunization with a CD3$^+$, WT31$^-$ cloned cell line derived from peripheral blood of a healthy adult individual. Cell fusions were performed according to a standard method described previously (2). Anti-Ti-γA is of the IgG2a subclass. BMA 031 mAb was kindly provided by Dr. Kurrle from the Behring Co. (Marburg, Federal Republic of Germany). This reagent recognizes a monomorphic determinant of the Ti α/β receptor (4). All other antibodies used in this study have been extensively characterized. Immunofluorescence (IF) experiments were performed on freshly drawn peripheral blood from healthy adult individuals. Nonadherent cells were treated for 30 min with either fluoresceinated anti-Ti-γA alone or anti-Ti-γA plus, individually, anti-CD2, -CD3, -CD4, -CD5, -CD6, -NKH1, -Ia mAb coupled to phycoerythrin (PE), kindly provided by the Coultronics Co. (Margency, France). For the comparative analysis of anti-Ti-γA reactivity in peripheral blood, we used OKT3 (Ortho Pharmaceuticals, Raritan, NJ) and BMA 031 (Behring Co.). 3.0×10^4 cells were analyzed in each sample on an Epics C instrument (Coulter Electronics, Inc., Hialeah, FL). Control reagents were FITC-IgG and PE-IgG.

Immunoprecipitations. F6C7 cells were surface labeled with ^{125}I using a standard lactoperoxidase method (2). After several steps of preclearing with Staphylococcus A suspension as well as irrelevant antibodies, specific precipitations were carried out for 6 h at 4°C with either anti-NKFi or anti-Ti-γA coupled to protein A–Sepharose beads. Samples were then retreated twice, for 4 h, with the same antibodies before overnight precipitation with the reciprocal mAb. SDS-PAGE was performed using 9% polyacrylamide gels either under nonreducing conditions or under reducing conditions after addition of 5% 2-ME.
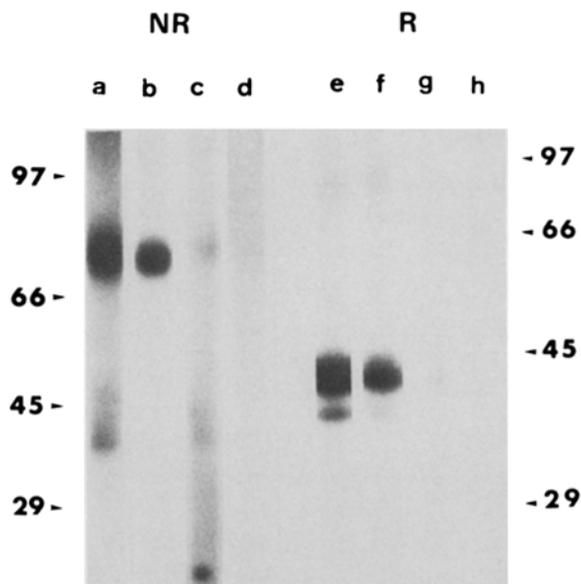
FIGURE 1. Immunoprecipitations of NKFi and Ti-γA molecules. Immunoprecipitations were performed as described in Materials and Methods section. Lanes a–d, nonreduced, lanes e–h, reduced after addition of 2-ME. Radiolabeled standards of various molecular masses were run in parallel with immunoprecipitates.

Results and Discussion

Anti-Ti-γA Recognizes the Same Molecule as Anti-NKFi. Mice were immunized with a CD3$^+$, WT31$^-$, NKFi$^-$, clone derived from peripheral blood of an adult donor. Among a large series of hybridoma supernatants, one reacted with the previously described F6C7 cells (1–3) and not with a conventional cloned T lymphocyte expressing an α/β heterodimer. The mAb secreted by this hybridoma, termed anti-Ti-γA, recognized an antigenic determinant comodulating with CD3 and NKFi on the surface of F6C7 lymphocytes (not shown). Thus, experiments were performed to compare the molecules immunoprecipitated from F6C7 cells by anti-Ti-γA and anti-NKFi. As previously reported, NKFi resolved in SDS-PAGE analysis as one 85 kD band under nonreducing conditions (Fig. 1 a) and as two bands at 44 and 41 kD under reducing conditions (Fig. 1 e). The anti-Ti-γA–precipitated material showed a virtually superimposable electrophoretic mobility (Fig. 1, b and f). Sequential precipitations confirmed the identity between NKFi and Ti-γA molecules because there was no material further recognized by anti-NKFi when samples were precleared with anti-Ti-γA (Fig. 1 c, nonreduced; lane g, reduced) and reciprocally (lane d, nonreduced; lane h, reduced). Because it was previously shown (3) that both the 44 and 41 kD NKFi bands are bound by a rabbit heteroantiserum specific for a 20-amino-acid peptide encoded by the first exon of the Cγ1 gene, we concluded here that anti-Ti-γA recognizes a TCR γ chain complex.

Anti-Ti-γA Defines a Subset of Peripheral Blood Lymphocytes. We tested anti-Ti-γA reactivity against a series of seven CD3$^+$, WT31$^-$, NKFi$^-$ cloned cell lines derived from peripheral blood of several adult donors. Five out of seven were found to be Ti-γA$^+$. Given this relatively broad distribution, it was possible that anti-Ti-γA would define a detectable subpopulation of peripheral blood lymphocytes. Analysis of 30 individuals revealed that ~3% of lymphocytes are Ti-γA$^+$,

TABLE I

Characterization of the Ti-γA Subset by Double-color Immunofluorescence Analysis

Antigens	A		B		C		D	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Ti-γA	6	3–15		—		—		—
CD2	77	56–85	5	2–15	7	3–17	89	36–99
CD3	71	65–77	6	2–15	9	3–21	97	92–99
CD4	41	24–51	<1	<1–1	<1	<1–2	5	<1–15
CD5	69	60–72	6	3–15	8	4–21	83	50–92
CD8	28	18–36	2	1–8	8	2–22	29	14–53
NKH1	12	3–18	<1	<1–3	8	1–24	13	6–30
Ia	6	3–9	<1	<1–1	7	2–14	7	2–19

Nonadherent lymphocytes were treated for 30 min with either fluoresceinated anti-Ti-γA alone or anti-Ti-γA plus, individually, anti-CD2, -CD3, -CD4, -CD5, -CD6, -NKH1, or -Ia mAb coupled to PE. Control reagents were FITC-IgG and PE-IgG. 3.0×10^4 cells were analyzed in each sample. Numbers represent ratio × 100, rounded to the nearest integer. A: Ratio between number of positive cells with one mAb and total number of cells analyzed. B: Ratio between number of double-fluorescent cells (that is positive with both anti-Ti-γA and the indicated PE-coupled antibody) and total number of cells analyzed. C: Ratio between number of double-fluorescent cells and total number of cells positive with PE-coupled antibody. D: Ratio between number of double-fluorescent cells and total number of cells positive with anti-Ti-γA.

with major differences from one donor to another ranging from <1 to 15% (data not shown).

Experiments that led to the discovery of γ proteins have been based on the observation that certain normal cloned fetal lymphocytes (1) as well as thymocytes (5) or lymphocytes derived from immunodeficient patients (6) reacted with anti-CD3 mAbs but not with anti-WT31, an mAb that recognizes a monomorphic determinant of Ti-α/β (7). It was shown that at least a fraction of these CD3⁺ Ti-α/β⁻ cells surface-express polypeptides (3, 5, 6, 8–10) encoded by T cell rearranging γ genes (for review, see reference 11). Such findings supported the view that a given T cell would carry either an α/β, or alternatively, a γ receptor complex. Normal peripheral lymphocytes were therefore tested by two-color immunofluorescence analysis with fluoresceinated anti-Ti-γA plus biotinylated BMA-031, revealed by avidin-PE. In all five donors tested, no double-labelled cells were detected, demonstrating that expression of TCR α/β and Ti-γA structures is mutually exclusive on peripheral lymphocytes (histograms from three distinct donors are shown in Fig. 2A). We then studied the relationship between the Ti-γA population and the well-characterized lymphocyte subsets defined by individual expression of CD2, CD3, CD4, CD5, CD8, NKH1, and MHC class II gene products. 10 healthy adults were tested. Two of them had only 1% of Ti-γA⁺ lymphocytes, which made a quantitative analysis of double-fluorescent cells rather inaccurate, in particular with second antibodies delineating additional minor subsets such as anti-CD8 or anti-NKH1; they have not been included in Table I, which summarizes the results obtained with the remaining eight individuals, for whom the average anti-Ti-γA reactivity measured on the lymphocyte population was 6%, ranging from 3 to 15%.

>94% of Ti-γA⁺ cells expressed CD2 in all donors but one (Table I). In this latter individual only a minority of Ti-γA⁺ lymphocytes reacted with Coulter clone CD2. Further studies will have to elucidate the significance of this observation. In seven donors, CD3 proteins were present on >95% of Ti-γA⁺ lymphocytes. In one case, anti-CD3 reacted with 92% of Ti-γA⁺ cells. This probably reflects a technical artifact such as, for example, partial antibody release rather
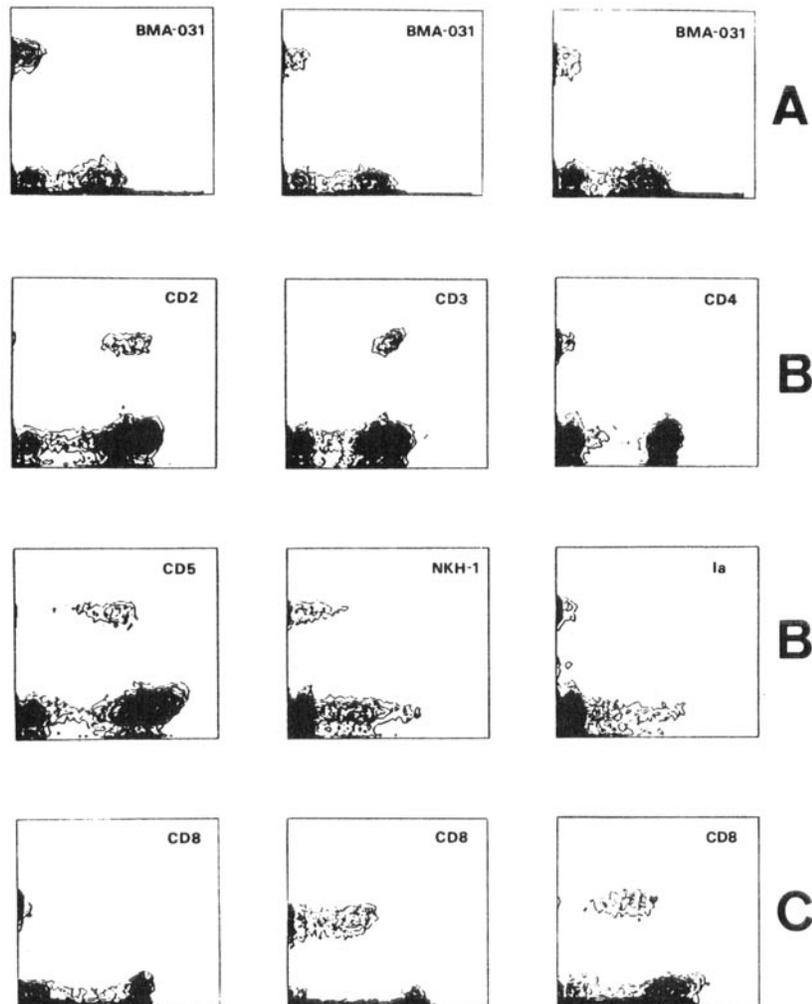
FIGURE 2.   Double-color immunofluorescence analyses. Immunofluorescence experiments were performed as described in Table I. Ordinate shows log_{10} green fluorescence obtained with fluoresceinated anti-Ti-γA antibody. Abscissa shows log_{10} red fluorescence obtained with the phycoerythrin-coupled mAb indicated in the figure. 3.0×10^4 cells were analyzed in each sample. A represents three distinct donors. B shows histograms obtained with a unique representative donor. C shows reactivity obtained in three distinct individuals with anti-CD8 antibody.

than a true surface expression of γ chains without CD3 proteins. Virtually all CD4$^+$ lymphocytes were found to be Ti-γA$^-$. There always was a small minority of Ti-γA$^+$ cells unreactive with anti-CD5. Moreover, as opposed to CD2 and CD3 (Fig. 2B), expression of CD5 was weak on Ti-γA$^+$ cells.

Analysis with anti-CD8 led to the most heterogeneous results. For example, two individuals had <20% of CD8$^+$ cells in their Ti-γA$^+$ population, while in one donor, a majority of Ti-γA$^+$ cells were CD8$^+$ (Table I). CD8 density was always weak on Ti-γA$^+$ cells (see Fig. 2C, which shows representative histograms obtained with three distinct normal individuals). Thus, an accurate delineation of at least one subset of peripheral lymphocytes expressing Ti-γ chains cannot

be based on the lack of expression of both CD4 and CD8 proteins. Anti-NKH1 (12) was found not to react with most Ti-γA$^+$ lymphocytes. When some reactivity was detected, it was weaker than that observed on CD3$^-$ NK cells. Finally, most Ti-γA$^+$ cells were found not to express class II MHC gene products, supporting the view that these lymphocytes are in a resting state.

Because a minority of CD3$^+$, WT31$^-$ clones that we have derived from peripheral blood are Ti-γA$^-$, it is most likely that the corresponding antibody does not detect all circulating lymphocytes with γ receptors. We have tried here to delineate the fraction of TCR γ^+ cells, which are recognized by anti-Ti-γA assuming, as a working hypothesis, that all CD3$^+$ TCR-α/β^- lymphocytes express at least one γ protein. For this purpose, we used the OKT3 mAb to detect cells expressing CD3 proteins and the BMA-031 mAb to detect cells expressing an α/β receptor. In the lymphocyte fraction isolated from an additional series of nine healthy donors, there were 6% (range, 1–12%) more OKT3$^+$ than BMA-031$^+$ cells, while the mean reactivity of anti-Ti-γA was 4% (range, 1–10%).

Further studies will have to elucidate the relationship between this predominant subpopulation of TCR-γ^+ lymphocytes and the remaining CD3$^+$ TCR-α/β^- Ti-γA$^-$ cells. In this regard, it should be of particular interest to identify the gene coding for the Ti-γA epitope (most likely, it is neither a C-γ1 or a C-γ2 segment, because our CD3$^+$ TCR-α/β^- Ti-γA$^-$ clones express disulfide-linked dimers as well as CD3$^+$ Ti-γA$^+$ cells). Furthermore, anti-Ti-γA mAb may help to study potential structural differences between CD3-associated γ complexes, which appear to include, in many cases, at least one additional still poorly characterized protein designated δ (or X) (6, 8–10, 13). Finally, the delineation of this novel subset should allow us to better characterize the biological role of TCR-γ^+ cells, and to determine whether protein products of T cell γ rearranging genes are involved as receptors directing functional activities such as, for example, non–MHC-restricted killing.

Summary

We have previously characterized a CD3$^+$ T cell receptor (TCR) α/β^- human fetal cloned cell line, termed F6C7, which surface-expresses a CD3-associated γ chain identified by anti-NKFi, an mAb with a restricted clonotypic reactivity. Here, we have produced an additional antibody, anti-Ti-γA, which recognizes a public epitope of the γ molecule defined by anti-NKFi. Ti-γA is present on ~3% of circulating lymphocytes with a wide range (1–15%) among 30 healthy individuals tested. Two-color immunofluorescence experiments performed with anti-Ti-γA and BMA 031 mAb (a reagent specific for the TCR-α/β receptor) showed that surface expression of Ti-α/β and Ti-γA is mutually exclusive. Moreover, it was found that most Ti-γA$^+$ cells are CD2$^+$, CD3$^+$, CD4$^-$, CD5$^+$, NKH1$^-$, HLA class II–negative. In contrast, the expression of the CD8 molecule on these T lymphocytes appears to be variable from one individual to another. Finally, we found that Ti-γA$^+$ cells represent a majority of peripheral lymphocytes that express CD3 proteins but not the TCR-α/β heterodimer. The delineation of this unique lymphocyte subset should help further studies on the biology of cells with a CD3-associated γ complex.

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