Surface Expression of Functional T Cell Receptor Chains Formed by Interlocus Recombination on Human T Lymphocytes

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Summary

Structural diversity of lymphocyte antigen receptors (the immunoglobulin [Ig] of B cells and the α/β or γ/δ T cell receptor [TCR] of T cells) is generated through somatic rearrangements of V, D, and J gene segments. Classically, these recombination events involve gene segments from the same Ig or TCR locus. However, occurrence of “trans” rearrangements between distinct loci has also been described, although in no instances was the surface expression of the corresponding protein under normal physiological conditions demonstrated. Here we show that hybrid TCR genes generated by trans rearrangement between Vy and D J3 elements are translated into functional antigen receptor chains, paired with TCR α chains. Like classical α/β T cells, cells expressing these hybrid TCR chains express either CD4 or CD8 coreceptors and are frequently alloreactive. These results have several implications in terms of T cell repertoire selection and relationships between TCR structure and specificity. First, they suggest that TCR alloreactivity is determined by the repertoire selection processes operating during lymphocyte development rather than by structural features specific to Vo~Vδ regions. Second, they suggest the existence of close structural relationships between γ/δ and α/β TCR and more particularly, between Vy and Vδ regions. Finally, since a significant fraction of PBL (at least 1/10⁴) expressed hybrid TCR chains on their surface, these observations indicate that trans rearrangements significantly contribute to the combinatorial diversification of the peripheral immune repertoire.

Lymphocyte antigen receptor diversity results from somatic DNA recombination between genetic elements originally separated on the chromosome, the V, D, and J segments. This recombination is mediated by specific enzymes, the lymphoid recombinases, that catalyze the joining of elements flanked by recombination signal sequences (RSS)¹ comprising highly conserved heptamer and nonamer motifs (1, 2).

Ig and TCR gene rearrangements are activated in lymphoid cells only, at a specific developmental stage and in a sublineage-dependent fashion, i.e., complete Ig rearrangements occur in B cells only, whereas complete TCR rearrangements occur in T cells only. Several observations suggest that specificity of the recombination process depends on two main parameters: first, on the ability of a given cell to activate its recombination machinery, as this property is specific to developing lymphocytes, and second, on the ability of a cell to render its TCR or Ig loci accessible to recombinases, as this accessibility is tightly regulated within a given lymphoid sublineage (for a review see reference 3).

Classically, the IgH and L chains on B cells and the TCR α and β or γ and δ chains on T cells are encoded by genes formed by elements belonging to the same locus. However, because rearrangements between elements belonging to any Ig or TCR locus seem to be mediated by the same recombination complex (4, 5), "trans" rearrangements between elements belonging to distinct loci could in theory occur provided that both loci are accessible to the recombinases at the same developmental stage. Hybrid IgH/TCR α chain genes have been described in leukemic cells by several groups about a decade ago (6–9). More recently, the occurrence of trans rearrangements between TCR γ and β elements has been demonstrated not only in leukemic cells but also in normal PBL (10–14). However, in the absence of any evidence for surface expression of the corresponding protein on normal lymphocytes, the physiological significance of these interlocus rearrangements has thus far remained unclear. Here we show that

¹ Abbreviations used in this paper: BLCL, B lymphoblastoid cell line; DP, CD4/CD8 double positive; RSS, recombination signal sequence.
hybrid TCR genes comprising VDJ elements from TCR-γ and β loci are translated into functional alloreactive TCR. These findings are discussed in terms of lymphoid lineage commitment, repertoire diversification, and TCR structure/specificity relationships.

Materials and Methods

Reagents and Cells. The following mAbs were used for flow cytometry analyses and immunomagnetic separations: 510 (pan δ [15]), 360 (anti-Vγ9 [15]), 23D12 (anti-Vγ2, 3, 4 [16]), 4A11 (anti-Vγ4 [17]), BMA031 (pan δ [18]), TCR δ (pan δ [19]), CγM1 (pan γ [20]), WT31 (anti-CD3 [21]), and F1 (anti-Vα2 [22]). F1 mAb was purchased from T-cell Diagnostics (Cambridge, MA). All the other mAbs were kind gifts from Drs. F. Romagné (Immunotech, Marseilles, France), M. Brenner (Harvard Medical School, Boston, MA), W. Tax (National Cancer Institute, Amsterdam, The Netherlands), A. Moretta (Istituto per la Ricerca sul Cancro, Genoa, Italy), and G. De Libero (Kantonspital, Basel, Switzerland).

The following B lymphoblastoid cells (BLCL) and mAbs used for functional assays were obtained from the VIIth HLA Workshop (New York, 1987): antibodies: D1.12 (anti-DR), Leu10 (anti-DQ), PL15 (anti-DP), and W6/32 (anti-HLA class I framework); BLCLs: RM (RML), IB (IBW9), BT (BTB), BO (BOLETH), SP (SP0010), and TU (TUBO).

Generation of γ/δ T Cell Clones and Lines. PBL from healthy donors were sorted, cultured, and cloned as previously described (23 and 24). In brief, cells were incubated with TCR V or C region-specific mAb for 45 min, washed once, and rotated for 4 h at 4 °C with magnetic beads coated with sheep anti-mouse Ig (Dynal, Oslo, Norway). After eight washes, bead-adherent cells were cultured in medium (RPMI 1640, 10% human serum, 1 mM L-glutamine, rIL-2, and leucogglutamin [0.5 μg/ml]), and irradiated PBL and BLCL. Beads were removed at day 5, cells were cultured further, and at day 14, they were subjected to a second immunomagnetic separation. After two more weeks of culture, cells were cloned by limiting dilution in culture medium and irradiated feeder cells. Growing colonies with a probability of monoclonality >95% were kept for further analysis.

Flow Cytometry Analysis and Comodulation Assays. Cells were phenotyped by indirect immunofluorescence as previously described (24). In brief, cells were incubated first with unconjugated V- or C-specific mAb for 30 min on ice, washed, and incubated with FITC-conjugated rabbit anti-mouse Ig antiserum for 30 min on ice. For single-color immunofluorescence, cells were resuspended in PBS after one washing, and analyzed by flow cytometry. For two-color immunofluorescence, cells were incubated for 10 min at room temperature with normal mouse serum, washed, then incubated for 30 min on ice with biotinylated mAb, washed, and finally, incubated for 30 min on ice with PE-conjugated streptavidin (Immunotech, Marseilles, France). After washings, cells were analyzed by flow cytometry on a FACScan® apparatus (Becton Dickinson & Co., Mountain View, CA) using the LYSYS II software.

Comodulation assays were performed as follows: cells were incubated with irrelevant or biotinylated 23D12 mAb for 30 min on ice, washed twice, and then incubated overnight in avidinated microwell plates (Immunotech; 10 cells per well). After two washes, cells were stained and analyzed by flow cytometry as described above.

Functional Assays. Proliferation and cytotoxicity assays were performed as previously described (24, 25). In brief, cytotoxic activity of T cell clones was measured by a standard 4-h 51Cr-release assay at 9:1 and 3:1 E/T cell ratios. Percent specific lysis was calculated as described previously (25). Proliferative activity of responder cells was estimated after a 48-h culture with irradiated BLCL followed by an overnight pulse with tritiated thymidine.

Amplification and Sequencing of TCR Transcripts. Preparation of T cell clone RNA, reverse transcription, amplification, and sequencing of Vγ-Cβ transcripts were performed as previously described (23) using Vγ1 (16) and Cβ (5' GGG AGA TCT CTG GTT CTG ATG GCT C) primers. TCR α transcripts were analyzed by anchored PCR as described earlier (26) using Co (5' GGT AGG ATC CTG TTT CAA AGC TTT TCT CGA CCA GC for reverse transcription; 5' CTT TGT GAC ACA TTT GTT TGA G for second step amplification and sequencing) and polyT (26) primers.

Results and Discussion

Characterization of a PBL Subset Recognized by both Vγ- and Cβ-specific mAb. In the course of the characterization of a mAb termed 23D12, which turned out to be directed against a determinant shared by Vγ2, Vγ3, and Vγ4 TCR variable regions (16), several 23D12+ polyclonal cell lines were generated after immunomagnetic sorting of PBL from healthy donors. Whereas the great majority of 23D12+ lymphocytes were recognized by the pan δ mAb #510 (15) and thus, presumably expressed a γ/δ TCR, a minor subset recognized
by the pan β mAb #BMA031 (18) but not by the pan δ mAb was reproducibly detected in most lines analyzed (Fig. 1 A and Table 1). The proportions of BMA031+ cells among 23D12+ T cells were generally 1%, and in about 1/4 of the cell lines studied, were >10% (Table 1 and data not shown). This indicated that cells with this peculiar phenotype were rather frequent under normal physiological conditions (>1/10^6 PBL considering that 23D12+ cells represent on average 1% of PBL [16]). To rule out a possible mAb cross-reactivity problem, additional staining experiments were performed on 23D12+BMA031+ T cell clones using a large panel of TCR V and C region-specific mAbs. Parts of 23D12+BMA031+ cells were recognized by a Vγ4-specific mAb (4A11 [17]) (Fig. 1 B and Table 1). Moreover, all of them were stained by the Cβ-specific mAb βF1 (20), as well as by the WT31 mAb (21), which is directed against a CD3ε epitope exposed on α/β but not on γ/δ T cells (data not shown). In contrast, 23D12+BMA031+ cells were recognized neither by the Cγ-specific mAb CγM1 (20), nor by the Cd-specific mAb TCRδ1 (19) (data not shown). Taken together, these results confirmed the presence of Vγ and Cβ epitopes and rule out expression of TCR γ and δ chains on 23D12+BMA031+ clones.

All 23D12+BMA031+ Clones Express Productively Rearranged TCR Genes Comprising Vγ and Cβ Elements. In light of recent studies demonstrating the occurrence of TCR chain gene rearrangements between TCR γ and β loci (10, 11), we suspected the presence of a hybrid TCR chain comprising Vγ and Cβ regions on the surface of 23D12+BMA031+ T cells. To test this directly, we performed PCR amplifications on mRNA derived from several 23D12+BMA031+ clones using primers specific to Cβ and to a DNA region shared by Vγ2, Vγ3, and Vγ4 elements. A DNA band of the expected size was amplified in all the clones tested using this pair of primers. Moreover, sequencing of the amplified cDNA demonstrated the occurrence of a productive trans rearrangement between Vγ and Jβ elements in all but one case (Fig. 2). Some but not all transcripts comprised a Dβ element, suggesting that γ/β trans rearrangements could occur both before or after partial DJβ rearrangements. An unusual hybrid sequence formed by a recombined VγJγ exon spliced to a Cβ exon was found in one clone (#71.13, Fig. 2). It has been previously proposed that this kind of transcript could result from a trans splicing between mRNA from rearranged TCR γ locus and unrearranged TCR β locus (11). The fact that such a structure was stably expressed in a T cell clone probably rules out a trans splicing event but rather suggests the occurrence of a secondary interlocus recombination following or preceding a normal intralocus VγJγ rearrangement. It is not clear at that stage whether this recombination event involves classical heptamer/nonamer RSS flanking the TCR V, D, and J elements or yet another signal sequence. In sup-

| PBL line | TCR-β among 23D12+ | TCR-δ among 23D12+ | Vγ4 among 23D12+α/β+ | CD4 among 23D12+δ* | CD8* among 23D12+γ/δ* | Vγ4 among 23D12+γ/δ* | CD4 among 23D12+γ/δ* | CD8* among 23D12+γ/δ* |
|----------|------------------|------------------|-----------------|---------------|----------------|-----------------|---------------|----------------|
| 67       | 1.3              | 98.8             | 99.8            | 0.6           | 99.9           | 20.2            | 0.6           | 0.0            |
| 69       | 6.0              | 94.1             | 87.1            | 98.3          | 4.5            | 20.0            | 0.0           | 1.6            |
| 70       | 3.0              | 97.3             | 57.0            | 19.4          | 80.3           | 47.3            | 2.8           | 0.0            |
| 71       | 1.9              | 98.2             | 47.4            | 18.8          | 53.2           | 29.1            | 0.5           | 1.0            |
| 72       | 0.0              | 99.6             | (−)            | (−)           | (−)           | 26.5            | 0.5           | 1.0            |
| 73       | 17.0             | 83.7             | 0.2             | 0.7           | 89.5           | 1.2             | 2.0           | 5.0            |
| 76       | 1.6              | 98.3             | 0.6             | 17.0          | 82.0           | 29.5            | 0.7           | 0.0            |
| 83       | 13.4             | 85.6             | 88.4            | 26.5          | 37.0           | 40.0            | 6.6           | 0.0            |
| 36       | 25.4             | 74.8             | 98.5            | 0.8           | 92.6           | 95.7            | ND            | ND             |
| 37       | 3.6              | 96.1             | 97.6            | 5.1           | 57.3           | 89.4            | ND            | ND             |
| 38       | 5.0              | 96.0             | 99.9            | 3.9           | 96.0           | 34.9            | ND            | ND             |
| 39       | 2.0              | 98.3             | 97.0            | 13.2          | 82.9           | 74.4            | ND            | ND             |
| Mean     | 6.7              | 93.4             | 70.3            | 18.6          | 70.4           | 42.6            | 1.8           | 1.2            |

23D12+ cultured PBL were recognized in a mutually exclusive fashion by the pan β (BMA031 [18]) and the pan δ (510 [15]) mAb. The percentage of Vγ4+ (4A11+ [17]) cells was significantly higher among 23D12+α/β+ than among 23D12+γ/δ+ lymphocytes (Wilcoxon matched pairs test, p = 0.02). Note that unlike 23D12+γ/δ+ cells, most 23D12+α/β+ cells expressed either CD4 or CD8 coreceptors.

Table 1. Frequency, Vγ Usage, and CD4/CD8 Coreceptor Expression by 23D12*BMA031+ T Cells Estimated by Flow Cytometry

* Percentage of CD8 bright.
† No 23D12+BMA031+ line could be derived from this individual. The percentage of Vγ4+, CD4+, and CD8+ cells among 23D12+α/β+ was estimated on sorted 23D12+BMA031+ PBL. The percentage of Vγ4+, CD4+, and CD8+ cells among 23D12+γ/δ+ lymphocytes was deduced from a combined analysis of 23D12+ and 23D12+α/β+ T cells.
pressed a TCR \( \beta \) chain gene comprising V\( \gamma \)4\( \gamma \)C\( \beta \) leading to a TCR \( \gamma \) chain comprising V3'4J\( \gamma \)P2 exon, normally spliced to a C\( \gamma \)2 exon (data not shown). Bank/DDBJ under accession numbers X81536-X81556.

amino acids. In the case of TCR \( \alpha \), most available Vc\( \gamma \) gene sequences were derived from cDNA clones. Therefore the assignment of a given junctional nucleotide or amino acid to V\( \gamma \) or to the N region could not be ascertained. (-) Not done. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers X81536–X81556.

ported the second possibility, the three examples of V\( \gamma \)4\( \gamma \)C\( \beta \) rearrangements described here and elsewhere (11) involved the same J\( \gamma \) segment (J\( \gamma \)P2), which would suggest the existence of a recombination hot spot located downstream of J\( \gamma \)P2, and presumably upstream of J\( \gamma \)2. These results raise the possibility that all hybrid TCR \( \gamma / \beta \) rearrangements are preceded by a common translocation event. In support of this, a preliminary Southern blot analysis of DNA from several T cell clones carrying either V\( \gamma \)4\( \gamma \)C\( \beta \), V\( \gamma \)D\( \beta \)B8C\( \beta \), or V\( \gamma \)B1C\( \beta \) rearrangements demonstrated the existence of a common nonassignable band hybridizing with the J\( \gamma \) probe p\( H \)60 (27 and our unpublished observations). An in-depth molecular analysis of several of these clones is currently underway. Taken together, these results suggest the existence of a primary recombination event possibly involving signal sequences distinct from the Ig and TCR RSS. If so, understanding its precise mechanism might provide clues on the etiology of diseases such as ataxia telangiectasia, where the frequency of trans rearrangements between V\( \gamma \) and C\( \beta \) is on average 10–50-fold higher than in normal individuals (11).

Hybrid TCR V\( \gamma \)C\( \beta \) Chains Are Paired to Classical Vc\( \gamma \)C\( \alpha \) Chains. In several polyclonal lines, a significant fraction of 23D12 * BMA031 * T cells was recognized by a V\( \alpha \)2-specific mAb (data not shown). Moreover, productive TCR \( \alpha \) chain gene transcripts were detected in all the 23D12 * BMA031 * clones studied (Fig. 2), suggesting that the hybrid TCR \( \beta \) chain was paired with a TCR \( \alpha \) chain. To formally prove this, we isolated a V\( \alpha \)2 * 23D12 * BMA031 * T cell clone after immunomagnetic sorting (Fig. 1 B), confirmed the presence of V\( \gamma \)4C\( \beta \) and V\( \alpha \)2C\( \alpha \) transcripts, and then, demonstrated a physical association between the V\( \gamma \)4C\( \beta \) and the V2C\( \alpha \) TCR chains by comodulation experiments using C\( \beta \)-, V\( \gamma \)-, and V\( \alpha \)-specific mAbs (Fig. 1 C).

Evidence for a Preferential Usage of Particular V\( \gamma \) but not V\( \alpha \) Region Genes by V\( \gamma \)C\( \beta \) Cells. A previous analysis of hybrid V\( \gamma \)C\( \beta \) gene rearrangements derived from normal PBL suggested a biased V\( \gamma \) gene usage in favor of V\( \gamma \)4 (11). Accordingly, the proportions of cells recognized by the V\( \gamma \)4-specific mAb 4A11 (17) were significantly higher among V\( \gamma \)C\( \beta \) + than among V\( \gamma \)C\( \alpha \) + cells (Table 1). Moreover, whereas we could derive cells expressing hybrid V\( \gamma \)C\( \beta \) chains using either V\( \gamma \)2, V\( \gamma \)3, or V\( \gamma \)4 genes from 19/20 PBL samples (Table 1 and data not shown), we were unable to isolate cells expressing V\( \gamma \)9C\( \beta \) chains despite numerous attempts. In contrast to V\( \gamma \), the V\( \alpha \) repertoire of 23D12 * \( \alpha / \beta \) T cells was not biased toward usage of a particular V\( \alpha \) region. Indeed, analysis of TCR V\( \alpha \) gene expression in polyclonal 23D12 * \( \alpha / \beta \) T cell lines using a panel of primers specific to the 29 V\( \alpha \) families demonstrated usage of distinct sets of V\( \alpha \) genes from one line to another (data not shown). Accordingly, productive V\( \alpha \) transcripts derived from the 23D12 * \( \alpha / \beta \) T cell clones studied comprised diverse V\( \alpha \) region genes (Fig. 2).

The process leading to underrepresentation of V\( \gamma \)9C\( \beta \) + and overrepresentation of V\( \gamma \)4C\( \beta \) cells irrespective of their V\( \alpha \) region is yet unclear. During development, rearrangements involving C-proximal V genes, such as V\( \gamma \)9, are activated at earlier stages than those involving C-distal V genes, such as V\( \gamma \)2, 3, and 4 (28). Perhaps V\( \gamma \)9 rearrangements occur at a stage when the whole \( \beta \) locus is not yet accessible to recombinases and thus, accessibility or combinatorial constraints might account for the absence of V\( \gamma \)9C\( \beta \) + cells. However, this cannot explain a biased V\( \gamma \) gene usage in favor of V\( \gamma \)4 because such a bias was observed among productive but not among nonproductive hybrid rearrangements involving V\( \gamma \) elements from the first V\( \gamma \) family (11). It is possible that the structure of some V\( \gamma \) regions is more or less compatible with stable conformation of the hybrid V\( \gamma \)C\( \beta \) chain and/or proper pairing with TCR \( \alpha \) chains. Also, biased usage of V\( \gamma \) but not V\( \alpha \) region by V\( \gamma \)C\( \beta \) cells might result from an in vivo selection by superantigens, whose recognition classically imposes constraints on one of the TCR V regions only (29). In this respect, some V\( \gamma \) regions, like most V\( \beta \) regions, show affinity for certain staphylococcal superantigens (30).

CD4 and CD8 Coreceptor Expression by V\( \gamma \)C\( \beta \) Cells. During their intrathymic development, \( \alpha / \beta \) and \( \gamma / \delta \) T cells follow two distinct differentiation pathways. Whereas most \( \gamma / \delta \) thymocytes remain CD4 * CD8 * throughout development, almost all \( \alpha / \beta \) T cells go through a CD4 *
CD8+ (double positive [DP]) stage (for a review see reference 31). Positive selection of these immature DP α/β thymocytes by self-peptide–MHC complexes leads then to their terminal differentiation into CD8+CD4− or CD8−CD4+ mature thymocytes (31). Because almost all 23D12+α/β T cells expressed either CD4 or CD8 coreceptors, unlike their γ/δ counterparts (Table 1), they probably differentiated from DP cells. Recent studies (32) have shown that transition to the DP stage tightly depends on the occurrence of a productive TCR β rearrangement. Therefore, the fact that CD4 and CD8 expression was induced after either VβIDβJβCβ or Vγ(Dβ)βJβCβ rearrangement within the precursor cell would indicate that transition to the DP stage is primarily dictated by the locus origin of the constant rather than the variable part of the rearranged gene, which is in accordance with recent observations made by others (33).

**Alloreactivity of VγCβ+ Lymphocytes.** Unlike γ/δ T cells, a large fraction of α/β T cells is reactive against polyvalent immunogenic MHC molecules. This allore cognition probably reflects the close structural relationships between the allomorphic MHC molecules recognized by mature α/β T cells and the self-MHC-peptide complexes against which immature α/β T cells have been positively selected (34). Analysis of the reactivity of polyclonal 23D12+α/β+ T cell lines toward six randomly chosen allomorphic B cells revealed strong proliferative (Fig. 3 A) or cytotoxic (Fig. 3 D) responses of most lines against one or more allomorphic cells. Moreover, alloresponses of some 23D12+α/β+ clones derived from these lines (Fig. 3 B) were blocked by the W6/32 mAb, which is directed against a constant part of classical MHC class I molecules (Fig. 3, C and D). Together with the presence of either CD4 or CD8 coreceptors on most 23D12+α/β+ T cell clones (Table 1), these results strongly suggest that the ligands recognized by 23D12+α/β+ cells are very similar to those recognized by “classical” α/β T cells.

These observations have several implications with respect to TCR selection processes and structural relationships between α/β and γ/δ TCR. Since most γ/δ cells (including 23D12+ cells) are CD4−CD8− and are not alloreactive (35), this would indicate that the same V region can participate in the recognition of distinct sets of ligands depending on the TCR chain to which it is associated. These results also suggest a close structural relationship between Vγ and Vβ regions, an assumption which is also supported by the fact that both V regions display superantigenic reactivity (29, 30), unlike Vα and Vδ regions. A nice parallel might be drawn between the relative interchangeability between Vγ and Vβ regions, which is supported by the present data, and the Vα/Vδ interchangeability, which is suggested by studies demonstrating the occurrence of VδCα rearrangements (36). It should be mentioned, however, that given the peculiar organization of the α/β locus, VδCα chains, unlike VγCβ chains, are formed by a classical intralocus rearrangement. In light of these observations, one could imagine the very same VγVδ combination to be associated with either Caββ or Cyδ regions. By comparing the antigenic specificity of these cells, it should be possible to directly test whether alloreactivity is a feature acquired through intrathymic TCR selection or whether it is dictated by germline motifs on Vα and/or Vβ regions.

**Concluding Remarks.** Here we have shown that a significant fraction of peripheral α/β T cells expressed surface hybrid receptors formed by interlocus recombination between TCR Vγ and (D)β families. Moreover, these cells displayed most features of mature α/β T cells and expressed functional alloreactive TCR. Hence, these results indicate that TCR trans rearrangements represent a novel mode of diversification that truly contributes to the combinatorial diversity of the peripheral immune repertoire.

**Figure 3.** Functional analysis of alloresponses mediated by 23D12+α/β+ T cells. (A) Proliferative response of polyclonal 23D12+α/β+ T cell lines after coculture with allogeneic BLCL. Polyclonal 23D12+BMA031+ lines (>99% pure) were cultured for 48 h in the presence of randomly chosen irradiated allogeneic BLCL, and pulsed for 18 h with tritiated thymidine ([3H]TdR). Shown are the proliferative responses (expressed in cpm x 10−3) of five cell lines (#69, #70, #71, and #76) against six randomly chosen allogeneic BLCL (TU, BO, BT, SP, and RM). Several other alloreactive T cell clones derived from this and other lines were obtained but are not shown here. (C) Blockage of allogeneic BLCL-induced proliferation of a 23D12+α/β+ T cell clone by anti-HLA mAb. The proliferative activity of a 23D12+BMA031+ T cell clone derived from line #76 (#76.3:CD8+; see Fig. 2) was estimated in the absence or presence of mAb directed against HLA framework determinants: W6/32 (anti-HLA I), D1.12 (anti-HLA DR), Leu30 (anti-HLA DQ), and PL15 (anti-HLA DP). Results are expressed in cpm x 10−3. (D) Blockage of 23D12+α/β+ T cell-mediated cytosis of an allogeneic BLCL by anti-HLA mAb. Cytolytic activity of 23D12+BMA031+ cells (line #70) against the allogeneic BLCL BO was estimated in the absence (−) or presence of anti-HLA framework mAb (see C). Results are expressed as percent specific target lysis (ordinate) calculated at 9:1 and 3:1 E/T ratios. Similar results were obtained with the 23D12+α/β+ T cell clone #70.1 derived from this line (Fig. 2 and data not shown).
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