Brief Definitive Report

IDENTIFICATION, AT THE GENOMIC LEVEL, OF AN HLA-DR RESTRICTION ELEMENT FOR CLONED ANTIGEN-SPECIFIC T4 CELLS

By GUNNAR PAULSEN, ERIK QVIGSTAD, GUSTAV GAUDERNACK, LARS RASK,* ROBERT WINCHESTER,‡ AND ERIK THORSBY

*From the Institute of Transplantation Immunology, The National Hospital, Oslo, Norway; the *Department of Cell Research, Wallenberg Laboratory, University of Uppsala, Sweden; and the ‡Orthopedic Institute, Hospital for Joint Diseases, New York*

T lymphocytes recognize antigen, together with molecules of the major histocompatibility complex (MHC), in the membrane of antigen-presenting cells (APC). This is MHC restriction. In man, the T4 subset of T lymphocytes recognizes antigenic epitopes together with histotopes (1) on the HLA class II molecules; DR, DQ, or DP (2, 3). The restriction elements have been difficult to define serologically; alloantigenic determinants on class II molecules show close but not complete concordance with the presence of a given restriction element (2).

Preliminary studies (4, 5) on some T4 lymphocyte clones (TLC) that are specific for epitopes on *Chlamydia trachomatis* and seem restricted by elements on DR molecules are closely associated but not identical to the serologically defined DRw53 (MT3) allospecificity. To better identify the restriction element for these TLC, DNA were prepared from a panel of APC donors some carrying the HLA class II restriction element, and were analyzed for restriction fragment length polymorphisms (RFLP) using a complementary DNA (cDNA) DRβ probe. A 9.3 kilobase (kb) band in a Hind III digest of DNA showed complete concordance with the ability of the APC to provide the restriction element for TLC. Thus, the elements on DR molecules restricting these antigen-specific T cells may be detected at the genomic level, though no serological definition is yet possible.

Materials and Methods

Cell Donors. The cloned T cells were derived from donor BS who had previously had an infection with *C. trachomatis*. Her HLA profile is HLA-A3; B7, 15; DR1, 4; DRw53, unknown; DQw1, 3; DPw4. Other cell donors were healthy staff members.

HLA Typing and Antibodies. All cell donors were HLA-ABC, -DR, and -DRw53 typed by highly selected alloantisera (6), including selected Ninth International Histocompatibility Workshop (9w) sera (7). The APC donors were also typed in indirect immunofluorescence with the monoclonal antibody (mAb) 109d6, which reacts with a DRβ chain, and detects a specificity closely correlated with, but not identical to the DRw53 specificity recognized by alloantisera (8, 9). The DPw1–4 specificities were determined by cloned alloreactive T cells (10).

T Lymphocyte Clones. Mononuclear cells from BS were stimulated with chlamydia antigen, T cell blasts were separated from nonresponding small T cells and cloned by

limiting-dilution technique (11). In cloning and expanding the TLC, we used irradiated autologous feeder cells and conditioned medium containing 20% T cell growth factor (TCGF) and 10% normal serum. The clones studied all expressed the OKT_4 marker.

Proliferative Assays. 10^4 cloned T cells were restimulated with chlamydia antigen at optimal concentrations (2×10^6 inclusion-forming units [IFU]/ml), using 10^4 irradiated non-T cells from different donors as APC, and in medium containing normal serum or an optimal dilution of HLA-specific mAb (5). Control wells received no antigen. The combinations were performed in triplicates for 60 h, then pulsed with [^{3}H]thymidine overnight. The results are expressed as mean cpm of triplicates, with SEM <20%.

DNA Isolation and Digestion by Restriction Enzymes. Peripheral blood (citrated) was drawn from 31 donors. 250–500 μg DNA was isolated from 20 ml blood, mainly as in reference 12. Samples (20 μl) containing 10 μg DNA were digested separately overnight by Hind III and Pvu II (25 U), as specified by the manufacturer (Boeheringer Mannheim, Federal Republic of Germany), followed by electrophoresis on 0.7% agarose gels (89 mM Tris-borate, 2.5 mM EDTA, pH 8.3; 40 V, 15 h). DNA was then transferred to nitrocellulose filters (Bio-Rad Laboratories, Richmond, CA) (13), and baked at 80°C for 2 h.

DNA Hybridization. Prehybridization, hybridization with a nick-translated [^{32}P]cDNA DRβ probe, and detection of radioactive bands has been described previously (14). A cDNA probe (pII-β-4) coding the whole DRβ chain (15) was used in the hybridization analysis.

Results and Discussion

Some of the chlamydia-specific clones from donor BS are restricted by elements closely associated with the DR1, DR4, or DPw4 class II allospecificities (16), while preliminary studies (4) suggested that TLC 4 and 37 from BS use restriction elements more closely correlated to DRw53.

A panel of APC donors was first typed with highly selected antisera, including those alloantisera that were found (7), during the most recent International Histocompatibility Workshop, to give the best definition of DRw53, as well as mAb 109d6, which detects a determinant closely associated with but not identical to DRw53 (8, 9). The results are given in Table I. TLC 4 and 37 are restimulated with chlamydia antigen and APC from donors 1–5 and 15–19 (donor 19 being the TLC cell donor BS), while APC donors 6–14 are unable to provide the correct restriction element. A close but not complete correlation is apparent between the ability of APC to provide the restriction element and the expression of the DRw53 allospecificity. Table I also shows that expression of the restriction element is closely but not completely correlated to reactivity of the APC with mAb 109d6. Donors 6 and 7, who carry the DRw53 allospecificity, fail to react with mAb 109d6, and lack the restriction element for TLC 4 and 37. In contrast, APC from donor 14 reproducibly reacted moderately with 109d6, but were unable to provide the restriction element. Other experiments (R. Winchester, unpublished observations) have also shown that 109d6 may rarely react with some DR1 expressing haplotypes. No correlation to any other class II allospecificities expressed by the APC was found, including DQw1–3 and DPw1–4.

TLC 4 and 37 were also studied in inhibition experiments, (5). The T cell clones were restimulated with chlamydia antigen and autologous APC in the presence of one of several mAb specific for different HLA molecules. Only mAb reactive with DR molecules, including mAb 109d6, significantly inhibited the TLC. Since mAb 109d6 was not able to inhibit the DR1-, DR4-, or DPw4-restricted, chlamydia-specific TLC from donor BS, these results further corroborate evidence that the restriction element for TLC 4 and 37 is present on DR molecules, and closely associated with those expressing the DRw53 allospecificity.

TABLE I

Restimulation of Two Chlamidia-specific TLC with Antigen and a Panel of APC

APC donor	[^{3}H]Thymidine uptake by TLC:		DR	Cytotoxicity‡ with APC of HLA type			
	4	37		9w734	9w743	9w735	109d6
	cpm						
1	3,088*	2,900	2, 7	3	3	3	2
2	2,986	3,832	5, 7	4	4	4	2
3	3,243	3,746	1, 7	3	3	3	3
4	3,587	2,506	4, 6	2	0	2	2
5	4,450	2,346	4, 8	2	1	3	3
6	118	152	7, 8	3	3	4	0
7	230	312	7, —	3	3	3	0
8	250	360	3, 8	0	0	1	0
9	188	228	2, —	0	0	1	0
10	114	120	3, 8	0	0	2	0
11	247	198	2, 5	0	0	0	0
12	242	232	6, —	0	0	0	0
13	264	362	1, 3	2	0	2	0
14	236	310	1, 2	0	0	0	1
15	1,781	2,342	2, 4	2	1	3	3
16	3,944	7,852	7, 8	4	4	4	3
17	3,863	3,522	4, 5	2	0	3	3
18	2,758	2,254	2, 7	4	4	4	2
19 (BS)	3,083	4,341	1, 4	0	0	2	2

* Mean cpm of triplicates, SE <20% of mean.

‡ Degree of cytotoxicity; 0, no cytotoxicity above background, 4, >80% killing of target cells.

Using selected restriction enzymes and cDNA probes that hybridize with class II genes, RFLP show close correlations to serologically detectable DR specificities (14, 17). DNA prepared from leucocytes of the panel of APC donors was digested with different restriction enzymes and examined with cDNA probes homologous to DRβ and DQβ genes. Using a DRβ probe (15), RFLP were detected that closely matched the expression of the serologically detectable DR specificities of the APC donors. For example, when DNA was digested with Pvu II, a band of ~16 kb was detected in donors 1–7 and 15–19 (data not shown), while a Hind III digest revealed a 7.8 kb band in the same donors (Fig. 1); i.e., among the APC donors expressing the DRw53 allospecificity (see Table I). Using Pvu II and the same DRβ probe, a band of ~15 kb was found (17) to be strongly associated with DRw53 (17).

Using Hind III digestion and the DRβ probe, a fragment of 9.3 kb was repeatedly detected in the DNA from APC donors 1–5 and 15–19; i.e., exactly those able to provide the restriction element for TLC 4 and 37 (Fig. 1). This fragment could not be found in DNA from donors 6–14; APC donors unable to provide the restriction element. To confirm this finding, a new panel of 12 APC donors was studied (Fig. 2). APC from seven of these donors were able to restimulate chlamydia-specific responses of TLC 4 and 37 (*R. epitope* + in Fig. 2). Only these seven donors also possessed the 9.3 kb fragment.

The molecular basis of the DRw53 specificity has not been fully determined. Recent evidence (9, 18) indicates that the DR molecules are encoded by a single α-chain gene and multiple β-chain genes within a haplotype. Secondly, DRw52 and DRw53 are allelic, but their β-chains seem to be determined by genes (DRβ_2) that are closely linked to but separate from the genes (DRβ_1) responsible for the specificities DR1-w14. Studies (4, 5) strongly suggest that the restriction element for TLC 4 and 37 is expressed by DR molecules. Also, using both highly selected alloantisera and RFLP analyses, the results demonstrate that the restriction

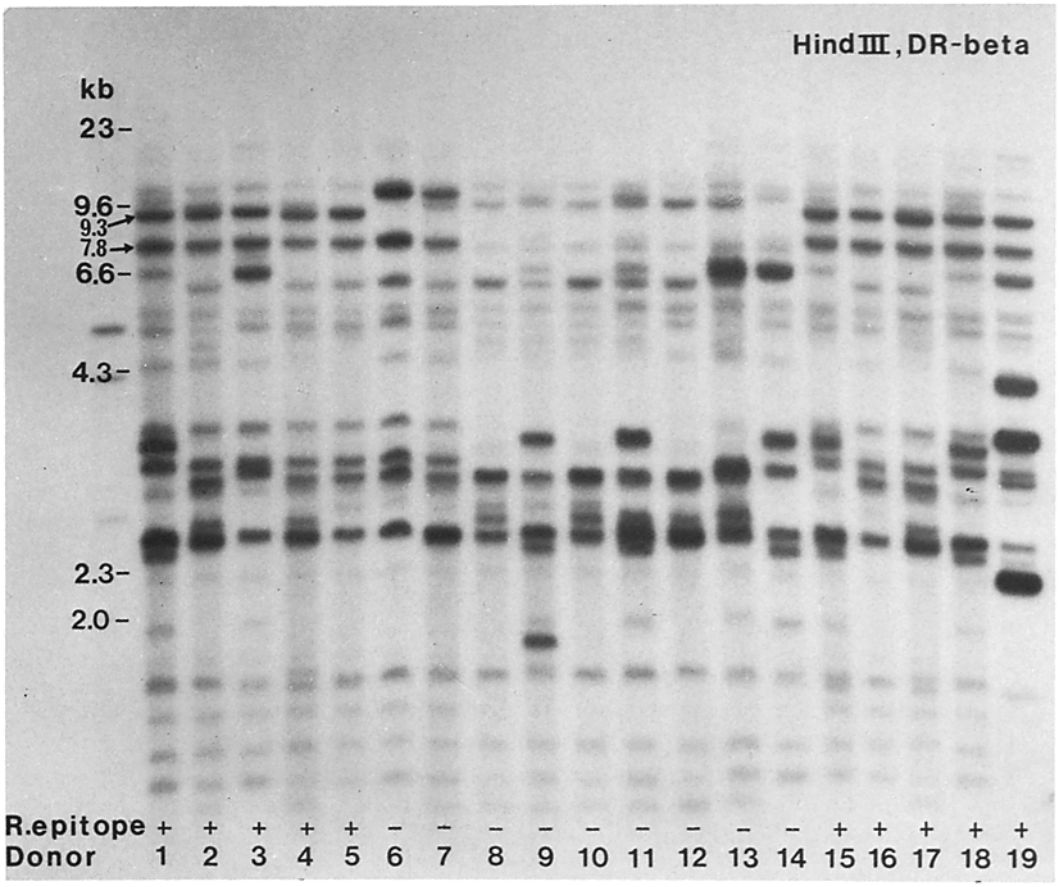

FIGURE 1. Hind III–digested genomic DNA prepared from APC donors 1–19 (Table I) electrophoresed on 0.7% agarose, transferred to nitrocellulose filter, and hybridized with a cDNA DRβ probe. Arrows indicate the 7.8 and 9.3 kb bands. *R. epitope* + designates presence of restriction element.

element for the cloned T cells is closely associated with, but not identical to the DRw53 specificity and the determinant recognized by mAb 109d6. Taken together, the results instead indicate that the class II DR molecules that provide the restriction element may often, but not always, also express the DRw53 allospecificity and determinants recognized by mAb 109d6. Alternatively, the gene (DRβ_3?) determining the restriction element for TLC 4 and 37 is closely linked, in disequilibrium, with the DRβ_2 gene, responsible for DRw53, and both genes may often also code for determinants recognized by mAb 109d6. The restriction element can presently only be detected by antigen-specific cloned T cells and RFLP analyses, and its precise structural nature must await further study.

The results demonstrate the discriminatory power of RFLP analyses. For the first time, a complete concordance between a particular HLA class II RFLP pattern and a restriction element for antigen-specific T cells has been detected. A similar correlation could not be found using a variety of alloantisera or mAb detecting other polymorphisms of HLA-DR molecules. Our results have important implications for studies on HLA and disease associations.

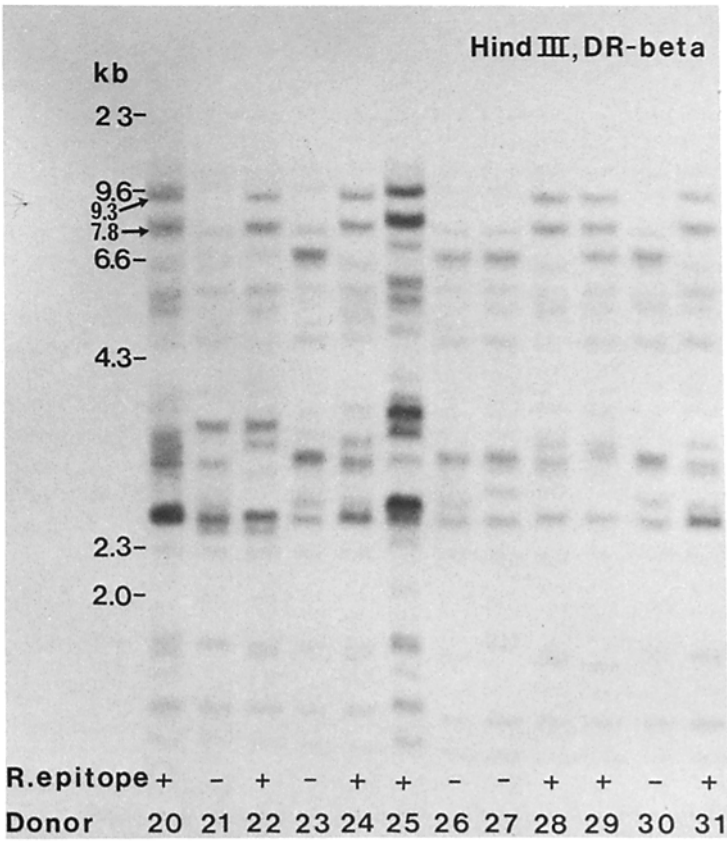

FIGURE 2. Hind III–digested genomic DNA from 12 additional APC donors, handled as in Fig. 1.

Summary

Two T4 cell clones (TLC) specific for antigenic epitopes on *Chlamydia trachomatis* were studied. Using a panel of allogeneic antigen-presenting cells (APC), both TLC were found to be restricted by HLA class II elements closely associated with, but not identical to the DRw53 specificity, as determined by highly selected alloantisera, a monoclonal antibody (mAb), 109d6, and confirmed on the DNA level by determination of restriction fragment length polymorphisms (RFLP) with a DRβ probe. Furthermore, HLA-DR-specific mAb, including 109d6, but not other HLA class II– or class I–specific antibodies inhibited the two TLC, strongly suggesting that the restriction element is expressed by a DR molecule. Using digestion with Hind III restriction enzyme and a DRβ probe, we found a complete concordance between the appearance of a 9.3 kilobase band and the ability of allogeneic APC to restimulate the T cell clones. Thus, the restriction element for these T cell clones appear to be expressed by DR molecules, but can, at present, only be detected at the genomic level.

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