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Diversity of anti-HLA-DR antibodies elicited by distinct anti-idiotypic monoclonal antibodies recognizing idiotopes co-expressed by the immunizing monoclonal antibody

S. M. MARIANI, E. A. ARMANDOLA & S. FERRONE *Department of Microbiology and Immunology, New York Medical College, Valhalla, New York, U.S.A.*

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SUMMARY

Analysis at the clonal level of the idiotypic network has identified differences in fine specificity between antigen-binding anti-anti-idiotypic (anti-anti-id) monoclonal antibody (mAb) and the original mAb as well as among antigen-binding anti-anti-idiotypic (anti-id) mAb. However, the diversity of humoral immune responses elicited by anti-id mAb recognizing idiotopes co-expressed on the immunizing mAb has not been analysed. Since this information may contribute to our understanding of the role of anti-id antibodies in the generation of diversity in the course of an immune response, we have compared the fine specificity and idiotype profile of two subsets of anti-HLA-DR mAb generated with the anti-id mAb F5-444 and F5-830. The latter mAb recognize idiotopes co-expressed in the antigen-combining site of the immunizing anti-HLA-DR1,4,w14,w8,9 mAb AC1.59. These investigations showed that: (1) the two subsets of anti-HLA-DR mAb overlap only partially in their reactivity patterns with HLA-DR$^+$ cells; (2) both subsets of anti-HLA-DR mAb recognize spatially close epitopes; (3) each subset of anti-HLA-DR mAb has unique reactivity patterns with soluble HLA-DRw16 and DRw17 antigens; and (4) each subset of anti-anti-id mAb displays a distinct idiotype profile. The subtle differences in the fine specificity and idiotype profile of the two subsets of anti-HLA-DR mAb suggest that anti-id antibodies may play a role in the generation of diversity in the course of a humoral immune response.

INTRODUCTION

The successful development of anti-id mAb in a number of antigenic systems (for review, see ref. 1) has rekindled interest in their potential use as surrogate antigens for active immunotherapy and in the functional and structural characterization of the idiotypic network.[2] Dissection at the clonal level of the immune responses elicited by anti-idiotypic (anti-id) monoclonal antibody (mAb) has identified at least two types of diversity. Antigen-binding anti-anti-idiotypic (anti-anti-id) mAb have been shown to display subtle differences in their fine specificity from the original antigen-binding mAb.[3–6] Furthermore, analysis of a panel of antigen-binding mAb elicited with anti-id mAb has detected diversity in their fine specificity and idiotypic profile among them.[7,8] However, diversity of the humoral immune responses elicited by distinct anti-id mAb recognizing idiotopes co-expressed in the antigen-combining site of the immunizing mAb has not, to the best of our knowledge, been analysed. This information may contribute to our understanding of the role of the idiotypic network in the generation of diversity in the course of a humoral immune response and may suggest criteria to select anti-id mAb to be used as immunogens. Therefore, in the present investigation we have compared the characteristics of the subsets of anti-HLA-DR mAb elicited with the structurally different syngeneic anti-id mAb F5-444 and F5-830. The latter two mAb recognize idiotopes co-expressed in the antigen-combining site of the immunizing mAb AC1.59[9] which defines a determinant shared by HLA-DR1,w8,9 antigens and by subtypes of HLA-DR4 and DR6 antigens, and poorly expressed by HLA-DRw16 and DRw17 antigens[7,10] (Fig. 1). This antigenic system was chosen for our investigations since the complexity of the determinant defined by mAb AC1.59 enhances the sensitivity of the detection of fine differences in the specificity of antigen-binding anti-anti-id mAb and the specificity of mAb AC1.59 mimics that of anti-HLA alloantisera.[11,12]

Abbreviations: anti-anti-id, anti-anti-idiotypic; anti-id, anti-idiotypic; PBS-T20, phosphate-buffered saline supplemented with 0·05% Tween 20.

Correspondence: Dr S. Ferrone, Dept of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595, U.S.A.

MATERIALS AND METHODS

Cell lines

HLA typed B-lymphoblastoid cell lines were grown in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS) and 2 mM L-glutamine.

Monoclonal antibodies

The mouse anti-id mAb F5-444 and F5-830 elicited with the syngeneic anti-HLA-DR1,4,w14,w8,9 mAb AC1.59 and the mouse anti-id mAb F3-C25 elicited with the syngeneic anti-HLA-DR,DP mAb CR11-462 were developed as described elsewhere.[9,13] The mouse anti-anti-id mAb SM/65, SM/75, SM/168, SM/180, SM/344, SM/466, SM/520, SM/524 and SM/549 elicited with the syngeneic anti-id mAb F5-444 and the mouse anti-anti-id mAb MA1/38, MA1/40, MA1/47, MA1/98, MA1/157, MA1/194, MA1/281 and MA1/285 elicited with the syngeneic anti-id mAb F5-830 were developed as previously described.[7,8] The anti-HLA-DR mAb KS9, the anti-HLA-DR,DQ,DP mAb KS16 and the anti-HLA class I mAb CR11-115 were characterized with a combination of serological and immunochemical methods (ref. 14; S. M. Mariani, E. A. Armandola and S. Ferrone, unpublished results).

IgG mAb were purified from ascitic fluid by sequential precipitation with caprylic acid and ammonium sulphate as described elsewhere.[15] The purity of IgG preparations was assessed by SDS–PAGE[16] and Coomassie Brilliant Blue staining. Only preparations without detectable contaminants were used.

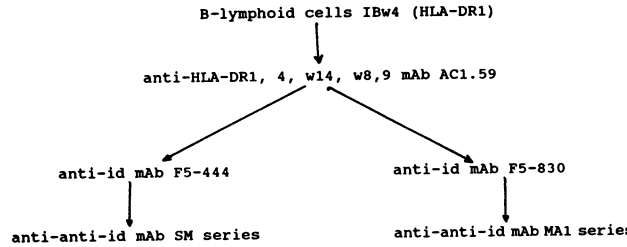

Figure 1. Idiotypic cascade in the HLA-DR system.

Antibodies were radiolabelled with Na-^{125}I using the chloramine T method.[17]

Serological assays

The binding assay of anti-anti-id mAb to HLA-DR$^+$ cells and the inhibition assay to map determinants recognized by anti-anti-id mAb on HLA antigens were performed in 96-well polystyrene microtitre plates (Falcon, Becton Dickinson, Oxnard, CA) as described elsewhere.[7,9] The double determinant immunoassay (DDIA) to measure the binding of soluble HLA-

Table 1. Comparison of the reactivity pattern with cell-bound HLA-DR antigens of the anti-anti-id mAb elicited with mAb F5-444 and F5-830*

Group	^{125}I-labelled mAb	LG2 DR1 Dw1	KAS011 DRw16 Dw21	COX DRw17 Dw3	LKT13 DR4 DwKT2	WALK DR4 Dw4	YALLUP DRw8 Dw8	KT12 DR9 Dw23
1	SM/65†	27·3	—§	4·0	11·6	6·9	7·2	26·3
	SM/549	113·8	20·2	20·9	103·4	16·2	78·5	40·2
2	SM/334	38·4	—	—	18·2	2·1	9·1	10·1
	MA1/38‡	21·4	—	—	27·2	8·9	16·9	8·6
	MA1/40	13·0	—	—	13·2	7·8	5·5	9·4
	MA1/47	5·4	—	—	26·6	13·1	3·5	9·2
	MA1/98	7·3	—	—	13·4	2·4	2·0	17·0
3	SM/75	16·4	—	—	19·3	6·8	—	8·1
	MA1/281	9·2	—	—	10·0	3·3	—	6·4
4	MA1/52	2·7	—	—	3·5	—	2·5	6·9
5	SM/168	6·1	—	—	4·4	—	3·5	—
6	MA1/157	9·4	—	—	6·4	—	—	4·5
	MA1/285	5·5	—	—	6·8	—	—	3·4
7	SM/466	4·5	—	—	4·2	—	—	—
	SM/520	4·5	—	—	1·6	—	—	—
	SM/524	3·9	—	—	4·2	—	—	—
5	SM/180	—	—	—	—	—	—	—
	MA1/194	—	—	—	—	—	—	—

* ^{125}I-labelled anti-anti-id mAb (5×10^5 c.p.m./well) were incubated for 2 hr at 4° with HLA typed B-lymphoid cells (2×10^5/well) in 96-well microtitre plates. Plates were then washed five times with PBS and dried. Bound radioactivity was measured in a γ-counter. Results are expressed as specifically bound c.p.m. $\times 10^{-3}/2 \times 10^5$ cells. All anti-anti-id mAb did not react with HLA-DRw11$^+$ JBUSH cells and with HLA-DR7$^+$ PLH cells.

† Anti-anti-id mAb of the SM series were elicited with mAb F5-444.

‡ Anti-anti-id mAb of the MA1 series were elicited with mAb F5-830.

§ Less than threefold background value. Background c.p.m. were determined in wells in which PBS was added to each mAb in the absence of cells.

Table 2. Mapping of the determinants recognized on the HLA-DR4$^+$ LKT13 cells by the anti-anti-id mAb elicited with mAb F5-444 and F5-830*

Cold mAb	^{125}I-labelled mAb							
	SM/65†	SM/75	SM/168	SM/334	SM/466	SM/520	SM/524	SM/549
MA1/38‡	57	60	100	100	50	100	68	81
MA1/40	76	92	100	100	100	100	86	100
MA1/47	57	89	100	100	55	100	63	100
MA1/52	35	45	56	90	39	50	59	52
MA1/98	57	97	100	100	100	100	68	100
MA1/157	44	51	63	83	100	100	50	74
MA1/194	—§	—	—	—	—	—	—	—
MA1/281	77	100	100	100	100	100	100	100
MA1/285	40	81	100	100	82	100	55	87

* ^{125}I-labelled anti-anti-id mAb ($1{\cdot}5 \times 10^5$ c.p.m./well) were mixed with 50 μl of purified cold mAb (100 μg/100 μl) and incubated with cultured B-lymphoid cells LKT13 (2×10^5 cells/well) for 2 hr at 4°in a 96-well microtitre plate. Plates were washed five times with PBS and dried. Bound radioactivity was measured in a γ-counter. Inhibition of the binding is calculated as per cent of the binding in the presence of the anti-HLA class I mAb CR11-115. Results are expressed as per cent of the maximal inhibition obtained with the homologous cold mAb.

† Anti-anti-id mAb of the SM series were elicited with mAb F5-444.

‡ Anti-anti-id mAb of the MA1 series were elicited with mAb F5-830.

§ Inhibition was less than 15%.

DR antigens by anti-anti-id mAb and the binding assay to anti-id mAb were performed in 96-well polyvinylchloride microtitre plates (Dynatech Laboratories, Alexandria, VA) as previously described.[7,18,19]

RESULTS

Comparison of the reactivity patterns with cell-bound HLA-DR antigens of the anti-anti-id mAb elicited with mAb F5-444 and F5-830

The reactivity patterns of the anti-HLA-DR anti-anti-id mAb with a panel of HLA-homozygous B-lymphoblastoid cell lines was compared using a high concentration of each ^{125}I-labelled anti-anti-id mAb in order to increase the sensitivity of the binding assay. The specificity of the binding was monitored using the anti-anti-id mAb SM/180 and MA1/194, elicited with mAb F5-444 and F5-830, respectively, which do not bind HLA-DR antigens. According to their reactivity patterns the two subsets of anti-anti-id mAb were classified into seven groups (Table 1).

The anti-HLA-DR mAb elicited with mAb F5-444 display the broadest range in their reactivity patterns: it spans from reactivity with all the HLA-DR specificities expressing the determinant defined by mAb AC1.59, to that restricted to HLA-DR1 antigen and to the HLA-DR4 subtype associated with DwKT2. On the other hand, the reactivity patterns of the anti-anti-id mAb elicited with mAb F5-830 range from reactivity with HLA-DR1, DRw8 and DR9 allospecificities and the two HLA-DR4 subtypes tested, to that restricted to HLA-DR1 and DR9 allospecificities and the HLA-DR4 subtype associated with DwKT2. Furthermore, the extent of binding to a given HLA-DR allospecificity of the anti-anti-id mAb elicited with mAb F5-444 varied up to 100-fold, while that of anti-anti-id mAb elicited with mAb F5-830 varied only up to fourfold.

Only a few of the anti-HLA-DR mAb from the two subsets show very similar reactivity patterns with HLA-DR$^+$ cells. Thus in group 2, mAb SM/334, elicited with mAb F5-444, and mAb MA1/38, MA1/40, MA1/47 and MA1/98, elicited with mAb F5-830, reacted with cell-bound HLA-DR1, DR4, DRw8 and DR9 alloantigens. The extent of binding of mAb SM/334 to HLA-DR1 antigens was higher than that of the four anti-anti-id mAb elicited with mAb F5-830. On the other hand, the extent of binding of mAb SM/334 to the other HLA-DR allospecificities was lower than that of at least one of the four anti-anti-id mAb elicited with mAb F5-830. In Group 3, mAb SM/75 and MA1/281, elicited with mAb F5-444 and F5-830, respectively, reacted with HLA-DR1 and DR9 antigens and the two subtypes of HLA-DR4 allospecificities. The extent of binding of mAb SM/75 to all HLA-DR allospecificities was higher than that of mAb MA1/281. On the other hand, Groups 1, 5 and 7 include only mAb elicited with mAb F5-444 and Groups 4 and 6 only those elicited with mAb F5-830.

This comparison shows that both anti-id mAb F5-444 and F5-830 induced a highly heterogenous anti-HLA-DR immune response. The extent of heterogeneity is greater among antibodies elicited by mAb F5-444 than among those elicited by mAb F5-830. It is noteworthy that there is only partial overlapping in the reactivity patterns with cell-bound HLA-DR antigens between the two subsets of anti-HLA-DR mAb. Only groups 2 and 3 include anti-HLA-DR mAb generated by anti-id mAb F5-444 and F5-830 even though the anti-HLA-DR mAb differ in their extent of binding to the distinct HLA-DR allospecificities.

Table 3. Comparison of the reactivity pattern with soluble HLA-DR antigens of the anti-anti-id mAb elicited with mAb F5-444 and F5-830*

mAb	LG2 DR1 Dw1	KAS011 DRw16 Dw21	COX DRw17 Dw3	LKT13 DR4 DwKT2	WALK DR4 Dw4	YALLUP DRw8 Dw8	KT12 DR9 Dw23
SM/65†	18·6	—	3·0	38·4	19·2	16·7	9·9
SM/75	4·7	—	—	7·3	5·3	2·9	4·6
SM/168	12·3	—	—	19·0	12·9	1·8	8·0
SM/180	—	—	—	—	—	—	—
SM/334	13·7	—	—	18·9	14·4	6·9	7·0
SM/466	4·2	—	—	6·1	4·8	0·8	1·4
SM/520	30·5	—	—	24·3	31·1	11·6	14·1
SM/524	11·3	—	—	12·2	11·9	7·2	6·3
SM/549	39·9	3·7	4·1	84·0	40·5	31·2	16·8
MA1/38‡	18·3	—	2·0	29·0	13·5	7·3	16·8
MA1/40	22·5	0·9	3·1	33·8	30·7	11·8	19·2
MA1/47	18·7	—	5·4	30·9	12·8	2·3	15·7
MA1/52	16·2	—	9·4	28·3	15·6	3·2	13·5
MA1/98	20·4	1·9	6·7	34·5	28·8	14·4	20·2
MA1/157	2·3	—	2·0	11·7	7·9	—	8·0
MA1/194	—	—	—	—	—	—	—
MA1/281	8·6	—	4·3	25·1	26·6	11·9	15·9
MA1/285	7·3	—	1·0	16·3	9·5	—	10·2

* Microtitre plates were coated with anti-anti-id mAb (125 ng/well). Following a 16-hr incubation at 4°, plates were washed three times with PBS-T20 and spent medium (100 μl/well) from HLA-DR$^+$ cells was added. After a 12-hr incubation at 4°, plates were washed three times with PBS-T20 and ^{125}I-labelled anti-HLA-DR mAb KS9 was added (2×10^5 c.p.m./50 μl/well). Incubation was continued for an additional 2 hr at room temperature. Plates were then washed five times with PBS-T20 and dried. Bound radioactivity was measured in a γ-counter. Results are expressed as specifically bound c.p.m. $\times 10^3$/well. Non-specific binding of ^{125}I-labelled mAb KS9 was measured in microtitre plates coated with the anti-id mAb F5-444. All the anti-anti-id mAb did not react with HLA-DRw11 and HLA-DR7 antigens present in the spent medium from cultures of HLA-DRw11$^+$ JBUSH cells and of HLA-DR7$^+$ PLH cells, respectively.

† Anti-anti-id mAb of the SM series were elicited with mAb F5-444.

‡ Anti-anti-id mAb of the MA1 series were elicited with mAb F5-830.

Mapping of the determinants recognized on HLA-DR$^+$ cells by the anti-anti-id mAb elicited with mAb F5-444 and F5-830

The spatial relationship between the determinant(s) recognized by the anti-HLA-DR mAb elicited with mAb F5-444 and F5-830 on the target antigen was analysed with an inhibition assay. All the anti-anti-id mAb, independently from the anti-id mAb used for their generation, reacted with spatially close determinants since all the anti-HLA-DR mAb elicited with mAb F5-830 inhibited the binding of the ^{125}I-labelled anti-HLA-DR mAb elicited with mAb F5-444 to the HLA-DR4$^+$ LKT13 cells (Table 2). The specificity of the inhibition is indicated by the lack of effect on the binding of the ^{125}I-labelled anti-HLA-DR mAb to LKT13 cells by the anti-anti-id mAb SM/180 and MA1/194 that do not bind HLA-DR antigens. The anti-HLA-DR mAb elicited with mAb F5-830 differed in their ability to inhibit the binding to target cells of those elicited with mAb F5-444. Monoclonal antibodies MA1/40 and MA1/281, and mAb MA1/52 displayed the highest and the lowest inhibitory activity, respectively. The anti-HLA-DR mAb elicited with mAb F5-444 differed in their susceptibility to inhibition by the other subset of anti-anti-id, with mAb SM/520 and SM/65 displaying the highest and the lowest susceptibility, respectively. The different inhibitory activity and susceptibility to inhibition displayed by the anti-anti-id mAb parallel those observed in cross-blocking experiments performed within each subset of anti-HLA-DR mAb.[7,8]

Comparison of the reactivity patterns with soluble HLA-DR antigens of the anti-anti-id mAb elicited with mAb F5-444 and F5-830

Since the anti-anti-id mAb within each subset had been previously shown to differ among themselves in their reactivity with soluble HLA-DR antigens,[7,8] the reactivity patterns of the two subsets of anti-anti-id mAb with soluble HLA-DR antigens were compared. As shown in Table 3, all the anti-anti-id mAb, excluding mAb MA1/157 and MA1/285, displayed a broader reactivity pattern with soluble than with cell-bound HLA-DR antigens, as they reacted with soluble HLA-DR1, DR4, DRw8 and DR9 antigens. Monoclonal antibodies MA1/157 and MA1/285 reacted with soluble HLA-DRw8 antigens only when used at high concentrations; the extent of binding was lower than that displayed by other anti-anti-id mAb. Representative results of the dose-dependent reactivity of anti-anti-id mAb with HLA-DRw8 antigens are shown in Fig. 2.

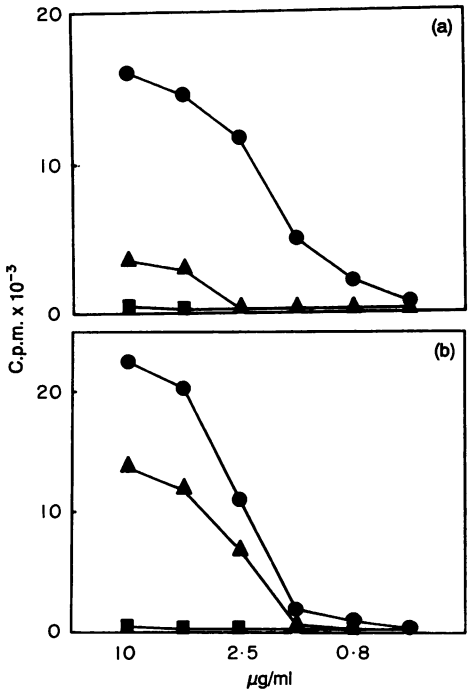

Figure 2. Dose dependent binding of soluble HLA-DRw8 antigens by anti-anti-id mAb elicited with mAb F5-444 and F5-830. Microtitre plates were coated with twofold dilutions of anti-anti-id mAb MA1/157 (a) and mAb SM/524 (b) (concentrations ranging from 10 μg/ml to 0·3 μg/ml). Following a 16-hr incubation at 4°, plates were washed three times with PBS-T20 and spent medium (100 μl/well) from the HLA-DRw8$^+$ cells YALLUP (▲) was added. After a 12-hr incubation at 4°, plates were washed three times with PBS-T20 and ^{125}I-labelled anti-HLA-DR mAb KS9 was added (2×10^5 c.p.m./50 μl/well). Incubation was continued for an additional 2 hr at room temperature. Plates were then washed five times with PBS-T20 and dried. Bound radioactivity was measured in a γ-counter. Results are expressed as specifically bound c.p.m. $\times 10^{-3}$/well. Non-specific binding of ^{125}I-labelled mAb KS9 was measured in microtitre plates coated with the anti-id mAb F5-444. Spent medium from cultures of HLA-DR4$^+$ LKT13 cells (●) and of HLA-DR7$^+$ PLH cells (■) was used as a positive and as a negative control, respectively.

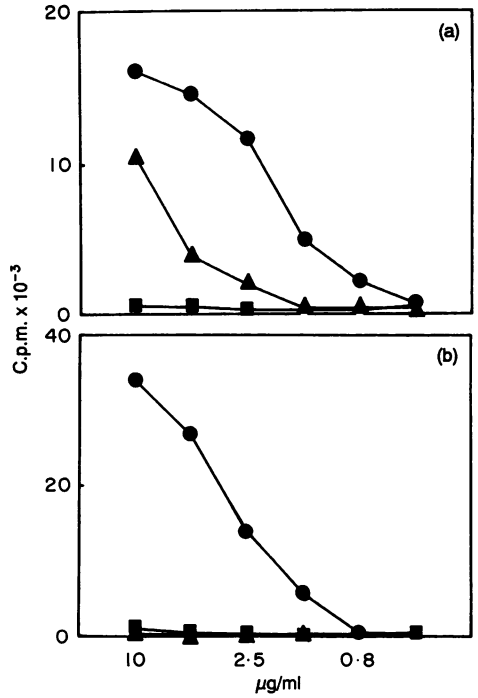

Figure 3. Dose-dependent binding of soluble HLA-DR3 antigens by anti-anti-id mAb elicited with mAb F5-444 and F5-830. Microtitre plates were coated with twofold dilutions of anti-anti-id mAb MA1/157 (a) and mAb SM/168 (b) (concentrations ranging from 10 μg/ml to 0·3 μg/ml). Following a 16-hr incubation at 4°, plates were washed three times with PBS-T20 and spent medium (100 μl/well) from the HLA-DR3$^+$ COX cells (▲) was added. After a 12-hr incubation at 4°, plates were washed three times with PBS-T20 and ^{125}I-labelled anti-HLA-DR mAb KS9 was added (2×10^5 c.p.m./50 μl/well). Incubation was continued for an additional 2 hr at room temperature. Plates were then washed five times with PBS-T20 and dried. Bound radioactivity was measured in a γ-counter. Results are expressed as specifically bound c.p.m. $\times 10^{-3}$/well. Non-specific binding of ^{125}I-labelled mAb KS9 was measured in microtitre plates coated with the anti-id mAb F5-444. Spent medium from cultures of HLA-DR4$^+$ LKT13 cells (●) and of HLA-DR7$^+$ PLH cells (■) was used as a positive and as a negative control, respectively.

The specificity of the binding of soluble HLA-DR antigens by the anti-anti-id mAb is indicated by the lack of reactivity of all mAb with soluble HLA-DRw11 and DR7 antigens. The latter are present in the spent medium of the HLA-DRw11$^+$ and HLA-DR7$^+$ cells JBUSH and PLH, respectively, at a level comparable to that of the other HLA-DR allospecificities as detected with the combination of the anti-HLA-DR,DQ,DP mAb KS16 and the ^{125}I-labelled anti-HLA-DR mAb KS9. Furthermore, ^{125}I-labelled anti-HLA-DR mAb KS9 did not bind to plates incubated with the spent medium from the B-lymphoid cell lines after coating with the anti-HLA class I mAb CR11-115 or with the anti-anti-id mAb SM/180 and MA1/194. The latter two mAb do not react with HLA-DR$^+$ cells.

It is noteworthy that the two subsets of anti-HLA-DR mAb displayed different reactivity patterns with soluble HLA-DRw16 and DRw17 antigens. All the anti-HLA-DR mAb elicited with mAb F5-830, that do not react with the HLA-DRw17$^+$ cells COX, reacted with soluble HLA-DRw17 antigens present in the spent medium of this cell line. Representative results of the dose-dependent binding are shown in Fig. 3. Within this subset of anti-anti-id mAb, mAb MA1/40 and MA1/98 reacted also with soluble HLA-DRw16 antigens. On the other hand, six of the anti-HLA-DR mAb elicited with mAb F5-444, that did not react with HLA-DRw17$^+$ cells, did not bind soluble HLA-DRw17 antigens even at the highest concentration of mAb used (Fig. 3). The remaining two anti-HLA-DR mAb, SM/65 and SM/549 reacted with both soluble and cell-bound HLA-DRw17 antigens. In addition, mAb SM/549 also reacted with soluble and cell-bound HLA-DRw16 antigens. The lack of reactivity with soluble HLA-DRw17 antigens of the majority of the anti-HLA-DR mAb elicited with mAb F5-444 is not due to an overall lower binding of soluble HLA-DR molecules by these mAb since they reacted with soluble HLA-DR4 antigens to an extent similar to that of the anti-HLA-DR mAb elicited with mAb F5-830. Representative results of this comparison are shown in Fig. 3.

These results indicate that the anti-id mAb F5-444 and F5-830 elicited two subsets of anti-HLA-DR mAb which differ in

Table 4. Comparison of the idiotypic profile of the anti-anti-id mAb elicited with mAb F5-444 and F5-830*

^{125}I-labelled mAb	Isotype	Anti-id mAb F5-444	F5-830	F5-963	F5-1419	F3-C25¶
SM/65†	G1	29·4	37·2	1·9	—	—
SM/75	G2b	44·3	48·9	1·9	—	—
SM/168	G1	37·6	46·7	16·1	—	—
SM/180	G1	26·7	40·4	3·5	—	—
SM/334	G2a	32·7	17·6	2·1	—	—
SM/466	G1	9·5	10·4	6·6	—	—
SM/520	G2a	29·3	28·1	—	—	—
SM/524	G2a	20·9	20·1	4·3	—	—
SM/549	G1	31·9	33·7	12·7	15·0	—
MA1/38‡	G1	56·6	79·5	—§	—	—
MA1/40	G1	14·5	24·1	—	—	—
MA1/47	G1	25·3	41·0	—	—	—
MA1/52	G1	19·3	51·7	1·4	88·1	—
MA1/98	G1	22·0	40·8	—	57·7	—
MA1/157	G1	33·7	85·2	7·3	—	—
MA1/194	G1	18·8	60·3	—	14·4	—
MA1/281	G1	39·2	56·8	8·6	46·8	—
MA1/285	G1	12·0	52·0	7·0	37·7	—

* Anti-id mAb-coated microtitre plates (10 μg/ml) were incubated with ^{125}I-labelled anti-anti-id mAb ($1{\cdot}5 \times 10^5$ c.p.m./well) for 2 hr at 4°. Plates were then washed five times with PBS-T20 and dried. Bound radioactivity was measured in a γ-counter. Results are expressed as bound c.p.m. $\times 10^{-3}$ c.p.m./well.

† Anti-anti-id mAb of the SM series were elicited with mAb F5-444.

‡ Anti-anti-id mAb of the SM series were elicited with mAb F5-830.

§ Less than 500 c.p.m.

¶ Anti-id mAb F3-C25 elicited with anti-HLA-DR, DP mAb CR11-462 was used as a specificity control.

their reactivity with cell-bound and soluble HLA-DRw16 and DRw17 antigens. Immunization with the anti-id mAb F5-444 generated anti-HLA-DR mAb that either recognize both cell-bound and soluble HLA-DRw16 and/or DRw17 antigens or do not recognize these alloantigens at all. On the other hand, the anti-id mAb F5-830 elicited anti-HLA-DR mAb that do not react with cell-bound HLA-DRw16 and/or DRw17 antigens, but bind soluble HLA-DRw16 and/or DRw17 antigens.

Comparison of the idiotypic profile of the anti-anti-id mAb elicited with mAb F5-444 and F5-830

The idiotypic profile of the two subsets of anti-HLA-DR mAb was compared using a panel of anti-id mAb elicited with mAb AC1.59. All the anti-anti-id mAb express the idiotopes defined by the anti-id mAb F5-444, F5-830, F5-1126 and F5-1336. On the other hand, the two subsets of anti-anti-id mAb differed in the expression of the idiotopes defined by the anti-id mAb F5-963 and F5-1419 (Table 4). All the anti-anti-id mAb elicited with mAb F5-444, excluding mAb SM/520, reacted with mAb F5-963, although with an up to eightfold difference in the extent of binding. In contrast, only four of the anti-anti-id mAb elicited with mAb F5-830 reacted with mAb F5-963 with an up to fivefold difference in the extent of binding. Conversely, five of the anti-anti-id mAb elicited with mAb F5-830 and one of the anti-anti-id mAb elicited with mAb F5-444 reacted with mAb F5-1419. In both subsets of anti-anti-id mAb the expression of the idiotopes defined by mAb F5-963 and F5-1419 did not correlate with their reactivity patterns with cell bound and soluble HLA-DR antigens, as they were expressed also by the anti-anti-id mAb SM/180 and MA1/194 which do not bind HLA-DR antigens.

DISCUSSION

In the present study, we show at the clonal level that there is diversity in the fine specificity and idiotypic profile between the anti-HLA-DR mAb elicited by the anti-id mAb F5-444 and F5-830, which define idiotopes co-expressed in the antigen-combining site of the immunizing anti-HLA-DR1,4,w14,w8,9 mAb AC1.59.[9]

Seven reactivity patterns with HLA-DR$^+$ cells were identified among the anti-HLA-DR mAb elicited with the anti-id mAb F5-444 and F5-830. The sharing of only two reactivity patterns by the anti-HLA-DR mAb generated with anti-id mAb F5-444 and F5-830 suggest that each anti-id mAb preferentially elicits a different subset of anti-HLA-DR mAb and that mAb with overlapping specificity represent only a minor component of the immune response. This conclusion is corroborated by the differential reactivity of the two subsets of anti-anti-id mAb with soluble HLA-DR antigens and by their differential expression of the idiotopes defined by the anti-id mAb F5-963 and F5-1419. These results indicate that distinct anti-id mAb, although recognizing idiotopes co-expressed on the immunizing mAb, may stimulate distinct B-cell subpopulations and generate immune responses with subtle differences in their characteristics. These findings have at least two implications, if they reflect a general phenomenon. The idiotypic network may play a role in the changes in the specificities of antibodies which have been observed in the course of a humoral immune response to mismatched HLA antigens.[20-22] Furthermore, within a panel of anti-id mAb elicited with the same mAb, the selection of an anti-id mAb to be used as a surrogate antigen may effect the efficacy of active specific immunotherapy, if the differences in the immune responses induced by distinct anti-id mAb have a functional significance.

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REFERENCES

1. Bona C.A. (ed.) (1988) *Biological Applications of Anti-idiotypes*, Vol. I. CRC Press, Boca Raton, FL.
2. Jerne N.K. (1974) Towards a network theory of the immune system. *Ann. Immunol. (Inst. Pasteur)*, **125C**, 373.
3. Percy A.J. & Harn D.A. (1988) Monoclonal anti-idiotypic and anti-anti-idiotypic antibodies from mice immunized with a protective monoclonal antibody against Schistosoma mansoni. *J. Immunol.* **140**, 2760.
4. Viale G., Flamini G., Grassi F., Buffa R., Natali P.G., Pelagi M., Leoni F., Menard S. & Siccardi A.G. (1989) Idiotypic replica of an anti-human tumor-associated antigen monoclonal antibody. Analysis of monoclonal Ab1 and Ab3 fine specificity. *J. Immunol.* **143**, 4338.

5. SHEARER M.H., LANDFORD R.L. & KENNEDY R.C. (1990) Monoclonal antiidiotypic antibodies induce humoral immune responses specific for simian virus 40 large tumor antigen in mice. *J. Immunol.* **145,** 932.
6. SHINJI T., ONO R. & FERRONE S. (1990) Fine specificity and idiotype diversity of a murine anti-HLA-DQw3 monoclonal antibody elicited with a syngeneic antiidiotypic monoclonal antibody. *J. Immunol.* **144,** 4291.
7. MARIANI S.M., ARMANDOLA E.A. & FERRONE S. (1991) Diversity in the fine specificity and idiotypic profile of mouse anti-HLA-DR monoclonal antibody elicited with the syngeneic anti-idiotypic monoclonal antibody F5-830. *J. Immunol.* **147,** 1322.
8. MARIANI S.M., ARMANDOLA E.A. & FERRONE S. (1992) Heterogeneity in the anti-HLA-DR immune response elicited by the anti-idiotypic monoclonal antibody F5-444. Analysis at the clonal level. *Transplantation* (in press).
9. PEROSA F. & FERRONE S. (1989) Murine antiidiotypic monoclonal antibodies that bear the internal image of HLA-DR allospecificities. *J. clin. Invest.* **84,** 907.
10. CREPALDI T., RICHIARDI P., MALAVASI F., CARBONARA A.O., IGARASHI M. & FERRONE S. (1985) The monoclonal antibody AC1.59 defines a new polymorphic determinant on HLA-DR molecules. *Tissue Antigens*, **26,** 25.
11. LEPAGE V., ALCALAY D., DOUAY C., MALLET C., LOISEAU P., DEGOS L., COLOMBANI M. & COLOMBANI J. (1985) Evidence for a new HLA class II determinant present on cells from HLA-DR1 and/or −DR4 individuals. *Tissue Antigens*, **25,** 96.
12. CARPENTER A.B., KAHL L.E., BARTKOWIAK C.D., DUQUESNOY R.J. & EISENBEIS C.H. (1988) Adult rheumatoid arthritis is associated with MC1, a new HLA-D encoded determinant. *J. Rheumatol.* **15,** 395.
13. PEROSA F. & FERRONE S. (1988) Syngeneic antiidiotypic monoclonal antibodies to the murine anti-HLA-DR,DP monoclonal antibody CR11-462. *Human Immunol.* **23,** 255.
14. TURCO M.C., DE FELICE M., CORBO L., MORRONE G., MERTELSMANN R., FERRONE S. & VENUTA S. (1985) Regulatory role of a monomorphic determinant of HLA Class I antigens in T cell proliferation. *J. Immunol.* **135,** 2268.
15. TEMPONI M., KAGESHITA T., PEROSA F., ONO R., OKADA H. & FERRONE S. (1989) Purification of murine IgG monoclonal antibodies by precipitation with caprylic acid: comparison with other methods of purification. *Hybridoma*, **8,** 85.
16. LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227,** 680.
17. GREENWOOD F.C., HUNTER W.M. & GLOVER J.S. (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89,** 114.
18. PEROSA F., KAGESHITA T., ONO R. & FERRONE S. (1989) Serological methods to detect anti-idiotypic antibodies. *Methods Enzymol.* **178,** 74.
19. RUSSO C., PELLEGRINO M.A. & FERRONE S. (1983) A double determinant immunoassay to quantitate human Ia antigens. *Transplant. Proc.* **15,** 57.
20. LEGRAND L., DAUSSET J. & RAPAPORT F.T. (1971) Serological and genetic studies of the HL-A system: results of host immunization within haplo-identical donor-recipient combinations. *Transfusion*, **11,** 233.
21. CURTONI E.S., SCUDELLER G., MATTIUZ P.L., SAVI M. & CEPPELLINI R. (1972) Anti-HL-A antibody evolution in recipients of planned transfusions. *Tissue Antigens*, **2,** 415.
22. FERRARA G.B., TOSI R.M., AZZOLINA G., CARMINATI G. & KISSMEYER-NIELSEN F. (1972) The production of anti HL-A cytotoxic antisera through planned immunization by intravenous injection of small aliquots of whole blood. I. Immunization of unrelated recipients. *Tissue Antigens*, **2,** 359.