# PRESENCE OF IgT-C AND I-A SUBREGION-ENCODED DETERMINANTS ON DISTINCT CHAINS OF MONOCLONAL ANTIGEN-SPECIFIC AUGMENTING FACTOR DERIVED FROM A T CELL HYBRIDOMA*

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Recent serological analyses suggest that the antigen-binding molecule of certain T cells carries determinants that were originally defined on the variable region ($V_H$)[1] of the immunoglobulin heavy chain. These include idiotypic markers linked to the immunoglobulin heavy chain gene cluster (Igh-1) (1, 2) and the framework determinants of $V_H$ (3–5). In addition, genetic evidence showed that the expression of a characteristic fine specificity of hapten recognition by T cells maps to the Igh-1 linkage group (4, 6). In accord, recent studies have reported that antigen-specific factors share idiotypic (7–10) and $V_H$ (5, 11, 12) determinants with antibodies. The constant region that is presumed to exist on the T cell antigen-receptor and antigen-specific factors, however, has been defined only negatively, in that Igh-1 constant-region determinants were not demonstrable (9–13).

Based on the partial relationship documented between the antigen receptors used by B and T cells, Owen et al. (14, 15) postulated that genes encoded the constant region of the T cell antigen receptor (IgT-C) may be located on chromosome 12, in close proximity to $V_H$ genes. Successful production of antiserum and monoclonal antibodies specific for T cell alloantigens was achieved by immunizing BALB/c mice with activated T cells from the Igh allotype congenic mouse, C.AL-20 (14–18). Three T cell markers that are linked to Igh-1, but are separable by genetic recombination, have been identified by monoclonal antibodies. The alloantigens $Tthy^d$ (17), $Tsu^d$ (14, 15), and $Tind^d$ (16) are expressed preferentially on thymocytes, peripheral Lyt-$2^+$ and Lyt-$1^+$ T cells, respectively, of mice possessing $Igh^d$ and $Igh^e$ allotypes. In vivo pretreatment of these mice with anti-$Tsu^d$ elicits suppression (15), while anti-$Tind^d$ causes either suppression or enhancement (16) of a primary antibody response. However, direct evidence that these alloantigens exist on an antigen-binding, biolog-

* Supported in part by a grant from the Ministry of Education, Culture and Science of Japan and grants R01 AI-15262 and R01 AI-17442 from the National Institutes of Health.

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; DNP, dinitrophenyl(ated); Ia, I region associated; Igh-1, immunoglobulin heavy chain gene cluster; IgT-C, constant portion of the T cell receptor for antigen linked to the Igh-1 locus on chromosome 12; KLH, keyhole limpet hemocyanin; NP, 4-hydroxy-3-nitrophenylacetyl; PFC, plaque-forming cells; PBS, phosphate-buffered saline; PV, *Bordetella pertussis* vaccine; TaF, augmenting T cell factor; TNP, trinitrophenyl; TsF, suppressive T cell factor; $V_H$, variable region of the immunoglobulin heavy chain.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/83/06/2110/11 $1.00
Volume 157 June 1983 2110–2120

ically functional molecule produced by T cells is lacking.

Previous publications from this laboratory reported the derivation of a T cell hybridoma made by fusion of antigen-binding T cells of A/J (H-$2^a$, Igh-$1^e$) mice with the thymoma BW5147 (12, 19). The cell line, designated FL10, can directly bind the antigen, KLH, and bears on its surface a structure to which antibodies against the framework of immunoglobulin $V_H$ cross-react (12). In addition, FL10 expresses a unique I region-associated (Ia) antigen and produces a characteristic antigen-specific, augmenting factor (TaF) that enhances the in vitro secondary response. TaF has been shown not to be helper T cell replacing, but acts on other T cells to induce augmention of the antibody response (13). The specificity of the TaF is twofold in that it can specifically bind to KLH and that augmentation is induced only when the responding cells are stimulated by haptenated KLH. This T cell hybridoma provided the opportunity to utilize monoclonal anti-$Tsu^d$ and anti-$Tind^d$ antibodies to determine directly whether TaF bears those well-defined allotypic determinants linked to Igh-1. In addition, the existence of monoclonal antibodies specific for I region products on T cells enabled us to study the relationship between Ia and IgT-C region determinants coexpressed on a functional T cell hybridoma. Evidence in support of the presence of IgT-C determinants on a molecule separate from that bearing T cell Ia antigen is presented in this communication.

## Materials and Methods

*Mice.* A/J (H-$2^a$, Igh-$1^e$), C3H (H-$2^k$, Igh-$1^j$), C57BL/6 (H-$2^b$, Igh-$1^b$), and B10.A (H-$2^a$, Igh-$1^a$) were purchased from Ohmura Experimental Animal Company (Kanagawa, Japan). C.AL-20 (H-$2^d$, Igh-$1^d$) mice, originally developed by Dr. Michael Potter at the National Institutes of Health, were propagated at the animal facilities of both the University of Tokyo and Tufts University School of Medicine. C.AL-20 mice are congenic to BALB/c except for an undetermined number of genes linked to the Igh-1 locus. BALB/cAnN animals were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA.

*Antigens.* Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA. The dinitrophenylated (DNP) conjugate was prepared by reaction with dinitrobenzene sulfonate under alkaline conditions. The molar substitution ratio was $DNP_{616}$-KLH (per $8 \times 10^6$ D KLH). *Bordetella pertussis* vaccine (PV) was purchased from Chiba Serum Institute, Chiba, Japan.

*Antisera.* The production and T cell specificity of anti-$Tsu^d$ antiserum has been described previously (14, 15). Briefly, BALB/cAnN females were immunized with concanavalin A (Con A)-stimulated lymphoblasts generated in vitro from normal C.AL-20 spleen cells. Polyvalent anti-mouse immunoglobulin antisera were obtained from rabbits and goats immunized with the γ-globulin fraction precipitated from normal mouse serum with ammonium sulfate and then emulsified in complete Freund's adjuvant.

*Monoclonal Antibodies.* Hybridomas that were produced by fusion of BALB/c anti-C.AL-20 splenocytes with the BALB/c myeloma, P.3U1 and which make monoclonal antibodies specific for T cell alloantigens linked to the murine Igh-1 locus were described previously (16–18). The characterization and antigen assignment of monoclonal anti-$Tsu^d$ (clone 13IIIB4, $IgG_1$ isotype), monoclonal anti-$Tind^d$ (clone 9IIIA2, $IgG_1$ isotype), and monoclonal anti-$Tthy^d$ (clone 17IIC6, IgM isotype) have been reported. A monoclonal antibody (14P) against an I region-encoded determinant expressed on a mature subset of T cells was reported previously (19). Clone 14P resulted from fusion of A.TH anti-A.TL spleen cells with the BALB/c plasmacytoma, P3-X63-Ag-8-653. The I region-controlled determinant recognized by 14P was mapped to the I-A subregion.

*T Cell Hybridomas.* The T cell hybridoma, FL10, was made by fusion of KLH-binding T cells of A/J mice with the AKR thymoma, BW5147 as previously described (12). FL10 expresses I region products and produces TaF, which binds to KLH and also augments the in vitro

secondary antibody response to DNP-KLH. An I-J$^k$-positive T cell hybridoma (7C3-13) with specificity for the hapten 4-hydroxy-3-nitrophenylacetyl (NP) was established by fusion of NP-binding T cells from B10.BR mice with BW5147. Clone 7C3-13 was selected for by testing the expression of I-J$^k$ and NP-specific suppressive activity, as will be described elsewhere (Suzuki et al., submitted for publication).

*Typing of T Cell Hybridomas by Immunofluorescence.* The T cell hybridomas ($5 \times 10^5$) were incubated for 1 h at 4°C in 50 μl of Dulbecco's modified Eagle's medium containing 0.2% bovine serum albumin, 0.04 M $NaN_3$, and conventional antiserum or monoclonal antibody in ascites form. Cells were washed three times and then stained with fluorescein-conjugated goat anti-mouse immunoglobulin for 1 h at 4°C. After washing three more times, the cells were examined under a Vanox fluorescence microscope (Olympus, Tokyo, Japan).

*Preparation of Monoclonal KLH-specific TaF.* The preparation of cell-free extracts containing functional T cell factors has been described elsewhere (20). Briefly, monoclonal TaF was prepared by disruption of FL10 hybridoma cells by freezing and thawing, followed by ultracentrifugation of the extract at 30,000 *g* for 1 h at 4°C. The extract was then frozen and maintained at −80°C until further use.

*Adsorption and Elution of TaF with Immunoadsorbents.* For the preparation of each immunoadsorbent column, 1 g of Sepharose 4B (Pharmacia Fine Chemicals) was coupled according to the method of Axen et al. (21) with 10 mg of KLH or the γ-globulin fraction of antiserum or ascites containing the $IgG_1$ myeloma, MOPC-31, or hybridoma antibody. The immunoadsorbents were packed in 5-ml syringes. The cell-free extract of FL10 equivalent to $10^7$ cells was applied to each immunoadsorbent column and incubated for 1 h at 4°C. The effluent equivalent to the void volume of the column was collected and the column was washed with a volume of cold phosphate-buffered saline (PBS) that was 10 times the column bed volume. The column-adsorbed material was then eluted with 3 ml of 0.175 M glycine-HCl buffer, pH 3.2. The eluates were neutralized immediately with 0.5 M potassium phosphate buffer, pH 8, and dialyzed against PBS at 4°C.

*Assay for Augmenting Activity.* The activity of TaF in untreated cell-free extracts of FL10 or immunoadsorbent fractions of those extracts were assayed by their addition to in vitro cultures of spleen cells from mice primed 4–6 wk previously with 100 μg DNP-KLH and $10^9$ PV. Spleen cells were cultured in a modified Mishell-Dutton system (22) in 24-well flat-bottomed dishes with a cell concentration of $4 \times 10^6$/ml of culture media and maintained in a humidified 5% $CO_2$/95% air atmosphere at 37°C. To assay TaF activity, the cell-free extract of FL10 was added to cultures on day 0. Because preliminary experiments showed the effective dose range to be rather narrow, two doses of the FL10 cell extract, equivalent to $5 \times 10^4$ or $10^5$ cells, were used. After 5 d, the numbers of anti-DNP IgG plaque-forming cells (PFC) were quantitated in a modified hemolytic plaque assay (23) by subtracting the number of direct (IgM) PFC from the total number of TNP-specific PFC that were developed with a polyvalent rabbit anti-mouse immunoglobulin antiserum. Target sheep erythrocytes were coupled with trinitrophenyl (TNP) according to the method of Rittenberg and Pratt (24).

*Data Analysis.* From the results of 3–4 individual wells, the mean and standard deviation of PFC for each group were calculated. Results of in vitro antibody-forming cell assays were statistically analyzed with a two-tailed Student's *t* test.

## Results

*Staining of an Augmenting T Cell Hybridoma with Monoclonal Antibodies against an Igh-1-Linked T Cell Alloantigen.* All strains of mice possessing the allotypes Igh-1$^d$ and Igh-1$^e$ were shown previously to express a determinant recognized by conventional anti-Tsu$^d$ antiserum (15). This conventional antiserum has at least two different specificities and monoclonal antibodies against each of these two alloantigens have been established (16, 18). Therefore, experiments were performed to determine whether or not the augmenting T cell hybridoma FL10, derived from A/J mice, expressed an antigen recognized by conventional anti-Tsu$^d$ antiserum and/or one of the monoclonal antibodies specific for T cell alloantigens linked to the Igh-1$^{d,e}$ loci. The reactivity of

these reagents with FL10 and, as a control, the T suppressor hybridoma 7C3-13 that originated from B10.BR mice, was assessed by indirect immunofluorescent staining. The results of one representative typing experiment are presented in Table I. Only FL10 was positively stained by both conventional anti-Tsu$^d$ antiserum and monoclonal anti-Tind$^d$. With higher dilutions of either reagent, the number of positively stained FL10 cells was greatly reduced (data not shown). Positive staining of FL10 with monoclonal anti-Tsu$^d$ or anti-Tthy$^d$ was not detected. Neither conventional anti-Tsu$^d$ antiserum, nor any of the monoclonal antibodies reacted with 7C3-13.

*Adsorption of Hybridoma-derived TaF by Immunoadsorbents Coupled with Anti-IgT-C Region Antibodies and Monoclonal Anti-I-A Antibodies.* Material extracted from FL10 was shown previously to enhance the secondary anti-DNP antibody response to DNP-KLH in the presence of KLH-primed helper T cells (12). Thus, experiments were done to determine whether cell-free, KLH-specific TaF from FL10 bears determinants recognized by monoclonal anti-Tind$^d$ antibody. In addition, since TaF produced by FL10 was already shown to be adsorbed by conventional anti-Ia antiserum (12), adsorption of the TaF activity with a monoclonal anti-I-A antibody was tested. This monoclonal antibody, designated 14P, was previously reported to be specific for a unique membrane determinant that is expressed by FL10 and controlled by the I-A$^k$ subregion, as determined by adsorption studies (19).

Columns were prepared using Sepharose conjugated with conventional antisera or the γ-globulin fraction of ascites from hybridoma-bearing mice and used as immunoadsorbents. Because both monoclonal anti-Tind$^d$ and anti-Tsu$^d$ antibodies are of the $IgG_1$ subclass, Sepharose coupled with the myeloma protein MOPC-31 served as a negative control. Cell-free material extracted from FL10 was applied to the various immunoadsorbents, and, after incubation, the filtrate and acid eluates were collected. The augmenting activity of these column preparations was assessed by their addition to cultures of DNP-KLH–primed spleen cells from A/J and C3H mice. The results of these experiments are shown in Fig. 1. Even in the absence of TaF, the response of A/J spleen cells was extremely high. Although there was a large difference in the magnitude of the response between the two strains, untreated extract from FL10 significantly enhanced the number of IgG anti-DNP plaque-forming cells in both

TABLE I

*Expression of the Igh-1$^{d,e}$-Linked Alloantigen, Tind$^d$ on the Cell Hybridoma, FL10**

| Reagent | Dilution | Percent of cells positively stained | |
|---|---|---|---|
| | | FL10 | 7C3-13 |
| Conventional anti-Tsu$^d$‡ | 1:20 | 30 | 3 |
| Monoclonal anti-Tsu$^d$§ | 1:10 | <1 | <1 |
| Monoclonal anti-Tind$^d$§ | 1:10 | 57 | <1 |
| Monoclonal anti-Tthy$^d$§ | 1:10 | <1 | <1 |
| NMS+‖ | 1:10 | <1 | <1 |

* T cell hybridomas were incubated with whole mouse serum or ascites and then indirectly stained with fluoresceinated goat anti-mouse Ig. Positively stained cells determined with fluorescence microscope.

‡ Whole serum.

§ Ascites from hybridoma-bearing mice.

‖ Normal mouse serum.

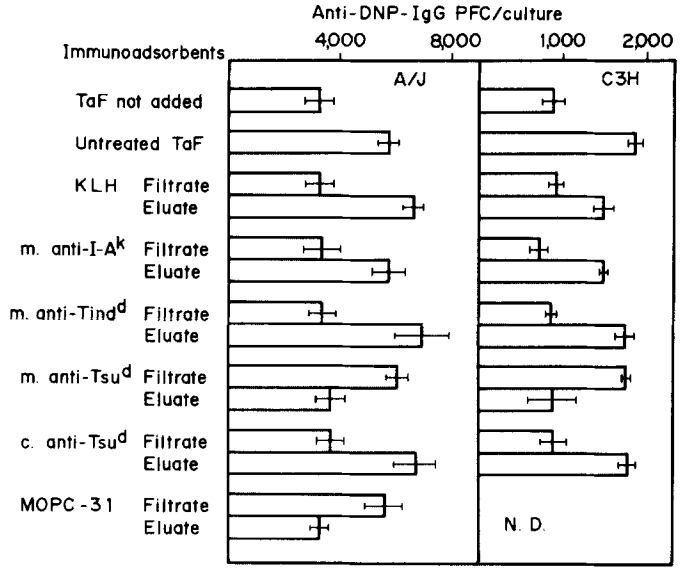


FIG. 1. Antigen-specific TaF from FL10 bears both Tind$^d$ and I-A$^k$ region-encoded determinants. $4 \times 10^6$ DNP-KLH primed spleen cells from A/J and C3H mice were cultured for 5 d in the presence of 0.5 μg of DNP-KLH with or without the addition of FL10 cell extract containing TaF. Filtrates or acid eluates were prepared from antigen- or antibody-coupled Sepharose columns to which FL10 cell extract was applied, as described in Materials and Methods. Column fractions were added to cultures on day 0. Bars represent the average number of IgG anti-DNP PFC from three to four culture wells and the standard deviation.

A/J and C3H spleen cells cultures ($P < 0.005$) when compared with the response of control cultures without TaF. While TaF was not detected in filtrates from columns coupled with KLH, column eluates significantly increased the response of both A/J and C3H ($P < 0.005$) in confirmation of the antigen specificity of the FL10 factor. FL10 extract passed through immunoadsorbents prepared with monoclonal 14P was no longer able to enhance the anti-DNP antibody response. Acid eluates from the 14P column significantly enhanced the in vitro antibody response ($P < 0.005$), indicating the recovery of TaF. The augmenting activity was also removed from the FL10 extract after passage through columns conjugated with conventional anti-Tsu$^d$ and monoclonal anti-Tind$^d$ antibodies and the activity to increase antibody responses was fully recovered in the acid eluates of these columns ($P < 0.005$ when compared with control responses). In contrast, adsorption of TaF by monoclonal anti-Tsu$^d$ or by MOPC-31 columns was not observed and the filtrates from these columns contained the original enhancing activity.

*Reconstitution of TaF Activity by Combining Column Filtrates: the Antigen-binding Chain of Monoclonal TaF Bears the Tind$^d$ Determinant.* Experiments were done to elucidate further the basic composition of the monoclonal TaF derived from FL10. Previous studies of antigen-specific TsF extracted from T suppressor hybridomas indicated that the TsF is composed of two distinct chains of which one binds antigen and the other bears antigenic determinants of the I-J subregion (25). Extract from suppressor cells contains the two chains, both in associated and nonassociated forms, although for effective suppression both chains are required. Thus, addition of column filtrates containing either chain alone to spleen cell cultures has no effect, but when the

filtrates are simultaneously added, suppression is induced. This study therefore examined whether the Tind$^d$ determinant is present on the antigen-binding chain rather than the I-A-bearing chain. Thus, TaF was incubated on columns coupled with KLH, monoclonal anti-I-A$^k$ (14P) or monoclonal anti-Tind$^d$. Filtrates from each column, equivalent to the extract of $5 \times 10^4$ or $10^5$ FL10 cells, or an equal mixture of two column filtrates were added to cultures of A/J and C3H spleen cells. In agreement with our previous findings, TaF activity was removed from filtrates of KLH, 14P, and monoclonal anti-Tind$^d$, but not monoclonal anti-Tsu$^d$, columns (Fig. 2). Reconstitution of augmenting activity occurred when the filtrates of the 14P column were combined with that of the KLH or monoclonal anti-Tind$^d$ column, but not when the KLH and anti-Tind$^d$ column filtrates were simultaneously added. Similar data were obtained in two other experiments using A/J mice as the responding strain. These results indicate that monoclonal TaF is a molecular complex consisting of two components: one chain bears I-A subregion-controlled determinants and the other chain possesses both antigen-binding activity and the Tind$^d$ determinant. FL10-derived cell extract contains the two chains in both associated and nonassociated forms. Similar to the findings for monoclonal TsF, both chains of the TaF must be present to induce an effect on the immune response.

*Genetic Restriction of Augmentation Induced by Monoclonal TaF.* The existence of both Igh-1-linked and I-A subregion-controlled determinants on the monoclonal TaF derived from FL10 raised the following question. Must the responding spleen cells share the I region haplotype and/or the immunoglobulin allotype with A/J mice, from which FL10 was derived, for FL10-derived TaF to successfully induce augmentation of the response? The results presented in Fig. 1 already demonstrated that monoclonal TaF from FL10 could equally enhance the response of A/J, the strain from which FL10 was derived, and C3H, which shares the I region, but not the Igh-

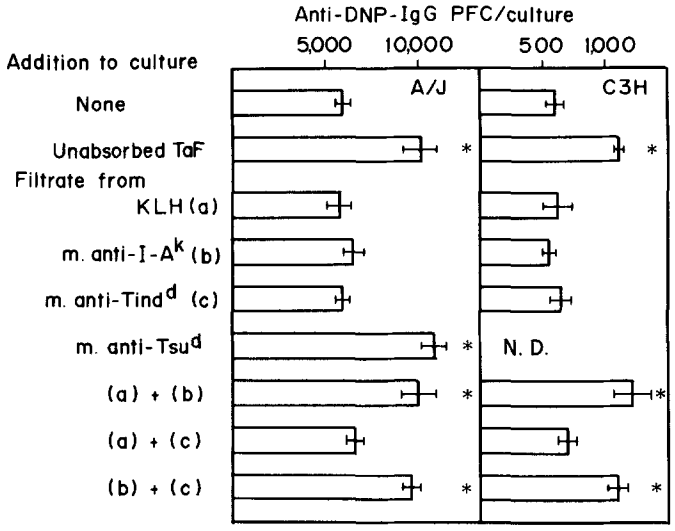


FIG. 2. Reconstitution of TaF activity by combining column filtrates. Spleen cell cultures were performed as in Fig. 1. FL10 cell extracts were applied to immunoadsorbent columns and the filtrates were collected. Column filtrates equal to the extract of $5 \times 10^4$ FL10 cells or an equivalent mixture of filtrates from two columns were added to in vitro cultures on day 0. (*) a significant difference in the response compared with that of control cultures without TaF ($P < 0.005$).

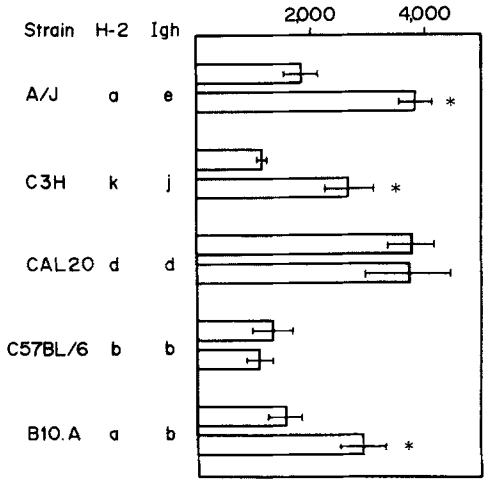


FIG. 3. Genetic restriction of augmentation by FL10-derived TaF. Spleen cell cultures were performed as in Fig. 1 with or without the addition of FL10-derived TaF. (*) a significant difference in the response compared with that of control cultures without TaF ($P < 0.005$).

1 locus with FL10. To elucidate further the genetic restriction, the ability of monoclonal TaF to augment the response of other strains of mice was tested. As shown in Fig. 3, induction of augmentation by TaF occurred only if the responding strain shared the I-A$^k$ subregion with FL10. Although C.AL-20 mice express the Tind$^d$ determinant, FL10-derived TaF did not induce augmentation of this strain's response, even when several different doses of TaF were tested (data not shown). Thus, the effect of TaF is not dependent on identity with the Igh-1 locus of the responder.

## Discussion

Originally, the T cell marker, Tind$^d$, was described as an alloantigen preferentially expressed on a mature, peripheral T cell population bearing the Lyt-1 differentiation marker (15). In vivo injection of monoclonal anti-Tind$^d$ antibody induced either enhancement or suppression of the response to sheep erythrocytes, but the exact biologic properties of the Tind$^d$-positive T cells were not further defined. Mapping of the Tind$^d$ determinant with Igh recombinant strains of mice limited the gene to that region of chromosome 12 to which Tsu$^d$ was assigned, between the end of the Igh-C cluster and a minor transplantation antigen, H(Ig) (15, 16). Because neither Tsu$^d$, nor Tind$^d$ is located among the heavy chain variable region genes, they are not genes encoding variable region framework or idiotypic determinants. Thus, the map position on chromosome 12 suggested that these T cell antigens were allotypic markers on the constant region of the T cell receptor for antigen.

Our study was initiated by the finding that conventional anti-Tsu$^d$, which contains both anti-Tsu$^d$ and anti-Tind$^d$ specificities, and monoclonal anti-Tind$^d$ antibody could stain the T cell hybridoma FL10. This Lyt-1$^+$2$^-$ T cell hybrid, originating from A/J (Igh-1$^e$) mice, was previously shown to produce TaF, which can bind to KLH and also enhance the antibody response to haptens coupled to KLH (12). In agreement with membrane fluorescence, our experiments found that TaF derived from FL10 bears the Tind$^d$ determinant. This was clearly demonstrated by the fact that the augmenting activity of TaF was completely adsorbed by columns coupled with

conventional anti-Tsu$^d$, which contains both anti-Tind$^d$ and anti-Tsu$^d$ activities, or with monoclonal anti-Tind$^d$, while filtrates from monoclonal anti-Tsu$^d$ columns retained the enhancing activity. In addition, TaF activity was bound to and eluted from Sepharose coupled with monoclonal anti-I-A antibody (14P) or with KLH. Thus, the presence of Tind$^d$ and I-A$^k$ subregion-controlled determinants on antigen-specific, monoclonal TaF were clearly shown. Additional results indicated that TaF was composed of two chains and that the Tind$^d$ determinant was present on the KLH-binding chain, rather than the I-A$^k$- bearing chain of the monoclonal TaF. The 14P column filtrate, although by itself devoid of activity, contained a component that could complement otherwise inactive filtrates from either KLH or monoclonal anti-Tind$^d$ columns for the restoration of augmenting activity. In contrast, a mixture of KLH and monoclonal anti-Tind$^d$ column filtrates failed to enhance the antibody response, suggesting that these column filtrates contained similar components of the monoclonal TaF. These findings indicate that Tind$^d$ is a marker on the constant portion of the antigen-binding chain associated with TaF. Further support for this conclusion is now being provided by the gel electrophoretic studies of radiolabeled TaF with monoclonal anti-Tind$^d$ and anti-I-A antibodies (S. Miyatani, K. Hiramatsu, P. Nakajima, F. L. Owen, and T. Tada, manuscript submitted for publication).

According to the original report by Owen et al. (14), Taniguchi and colleagues prepared similar conventional and monoclonal antibodies against Igh-1$^b$-linked T cell alloantigens (26–29). They found that certain monoclonal reagents were specific either for antigen-binding chains of suppressor or augmenting factors, indicating that functionally diverse T suppressor and T augmenting cells utilize different T cell receptor isotypes (29). The relationship of the Igh-1$^b$ T cell alloantigens with respect to Tind$^d$ and Tsu$^d$ remains to be determined.

All these data indicate that a region of mouse chromosome 12 encodes at least a portion of the constant region genes that comprise the antigen-binding structure of T cells. The total number of peripheral T cells recognized by these reagents, however, is rather small (14, 15, 18). This suggests that other T cell isotypes mapping to this region may be discovered. The mature T cell isotypes defined thus far appear to be expressed by T cell subsets that can bind antigen in the absence of MHC products (12, 20). Tests of a panel of monoclonal antibodies specific for Tthy$^d$, Tind$^d$, and Tsu$^d$ found that none reacted with cytotoxic effector or MLR-reactive T cells (G. M. Spurll, M. Frye, L. Riendeau, A. Finnegan and F. L. Owen, manuscript in preparation). Furthermore, Tind$^d$-positive and Tsu$^d$-positive cells are ontogenetically related in that Tthy$^d$-positive thymocytes are precursors of both of these mature T cell subsets (30). The antigen receptors used by this family of regulatory T cells may be distinct from those used by the majority of T cells, which see antigen in the context of H-2 molecules. Only further attempts to produce monoclonal anti-IgT-C antibodies and the generation of probes to identify IgT-C genes at the DNA level will provide answers.

It is well documented that the biologic functions of conventional TsF and TaF are governed by genetic restrictions imposed by the I region (13, 31, 32), and I-J and I-A subregions, respectively. Our findings show that augmentation of the secondary response induced with FL10-derived TaF also occurred only if the responding cells shared the Ia$^k$ region. Despite the presence of the Igh-1-linked determinant, Tind$^d$, on TaF, the induction of augmentation did not occur if the responding cells were only

Igh-1 compatible.

The existence of different IgT-C region isotypes and their association with different I region-controlled products prompts speculation as to the role each molecule plays in the regulatory activity of T cell factors. Both TaF and TsF activate a second type of cell, that is effective in regulatory circuits, either enhancing or suppressing the immune response (12, 13, 31, 33). Thus, each type of factor must fulfill at least two basic functions: the selection of the secondary acceptor cell and the activation of that cell. Future questions to be addressed are: which function (or functions) is performed by the IgT-C isotypic determinants on the antigen-binding chain vs. that performed by the I region-controlled chain, and further, how are these functions accomplished?

## Summary

Monoclonal antibodies specific for mouse T cell alloantigens, Tind$^d$ and Tsu$^d$, linked to the Igh-1 locus on chromosome 12, were used to directly define the antigen-binding molecule produced by a cloned hybridoma. The T cell hybridoma, FL10, was established from antigen-binding T cells of A/J mice. FL10 produces an antigen-specific augmenting T cell factor (TaF) that bears a unique I region-controlled determinant (I-A) and has antigen-binding capacity. The Tind$^d$, but not the Tsu$^d$, determinant was detected on the surface of FL10. The presence of both Tind$^d$ and I-A subregion-controlled determinants on FL10-derived TaF was directly demonstrated by the adsorption of TaF with immunoadsorbents prepared with monoclonal antibodies. The Igh-1-linked T cell alloantigen, Tsu$^d$, was not found on TaF. Further experiments indicated that Tind$^d$ is present on the antigen-binding polypeptide chain and not on the second chain bearing the I-A determinant. Despite the presence of the Tind$^d$ determinant on hybridoma-derived TaF, augmentation induced by TaF was restricted by the H-2 type of the responding mice and not by the Igh-1 allotype.

The excellent assistance of Ms. Yoko Yamaguchi in the preparation of this manuscript is greatly appreciated.

*Received for publication 7 December 1982 and in revised form 14 March 1983*

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