Brief Definitive Report

CD4+ MURINE T CELL CLONES THAT EXPRESS HIGH LEVELS OF IMMUNOGLOBULIN BINDING BELONG TO THE INTERLEUKIN 4-PRODUCING T HELPER CELL TYPE 2 SUBSET

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For each of the five H chain classes of Ig in the mouse, there is a corresponding Fc receptor (FcR) that can be detected on T and B lymphocytes (1). The five major classes of FcRs are constitutively expressed on normal, resting murine B lymphocytes but, with the possible exception of the IgG FcR on γ/δ T cells, FcRs are present at very low levels, or are not detected, on normal, resting murine T lymphocytes (1). Interestingly, activation with anti-T3 mAb induces the expression of FcRs on a fraction of normal murine T lymphocytes (2).

In principle, the display of Ig-recognizing molecules on activated T cells provides potential mechanisms for immunoregulatory interactions between T and B cells. Based on their patterns of lymphokine production, subsets of CD4+ murine T cells have been categorized into subsets designated Th1 and Th2 (3). Th1 cells produce IFN-γ, lymphotoxin, and IL-2, and promote delayed-type hypersensitivity reactions, lymphocytic inflammatory responses, and IgG2a antibody responses. Th2 cells produce the B cell growth factor IL-4 and the B cell differentiation factors IL-5 and IL-6 that promote IgG1, IgE, and IgA antibody responses.

The functional heterogeneity of murine CD4+ T cell subsets with regard to B cell helper functions prompted us to examine activated CD4+ T cell subsets for their expression of FcRs. We studied 12 Th1 and eight Th2 clones for the specific binding of antibodies of each of the isotypes IgM, IgD, IgA, IgG, and IgE, and found that the Th2 clones displayed high levels of up to four classes of FcRs, while the Th1 clones expressed low or undetected levels of FcRs.

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Materials and Methods

Proteins. Monoclonal mouse Igs were purified from MOPC315 (IgA), MOPC1017 (IgD), MOPC1033 (IgD), MOPC104 (IgM), hybridoma A3B1 (IgE), and hybridoma 2.4G2 (rat anti-mouse FcγII). Purified IgA, IgD, and 2.4G2 were biotinylated as described (4). Purified IgE and IgM were conjugated with FITC as described (4).

Cells. The designation, antigen specificity, and H-2 background of the $CD4^+$ T cell clones are listed in Table I. FcR expression was determined 7–14 d after boosting with nominal antigen presented on irradiated syngenic spleen cells. T cell clones were routinely screened for mycoplasma and were consistently negative. The L (16, 17, 18) and the GL (15, 17, 18) series of clones and clones L2, PL3, M6.6, and AD9 were isolated and characterized by Gajewski et al. (5), and were maintained in DMEM supplemented with 5% FCS and 10 U/ml Il-2. All the other T cell clones were maintained in RPMI supplemented with 10% FCS and 15% rat spleen cell Con A supernatant.

Detection of Fc Receptors. 5×10^6 T cells were incubated at room temperature for 30 min in HBSS/bovine calf serum containing purified IgM, IgA, IgD, IgE, or 2.4G2. Based on preliminary titrations using receptor-expressing normal and cloned lymphocytes and lymphoid tumor cell lines, the Ig concentrations used were at or above the saturating dose for the corresponding FcR. T cell-bound biotinylated Ig was detected by incubation for 30 min at room temperature with 5.0 μg/ml Avidin-FITC (Vector Laboratories, Inc., Burlingame, CA). Control cells were incubated with Avidin-FITC alone. Cells were washed three times with media, fixed in 2% paraformaldehyde in PBS (pH 7.2), washed, and analyzed in a flow cytometer (440; Becton Dickinson & Co., Mountain View, CA). The data are expressed as Δ mean fluorescence, a logarithmic value, where the background (mean fluorescence channel of cells plus avidin-FITC alone) was subtracted from the mean fluorescence channel value for each experimental sample. Compensation for autofluorescence was carried out as described (6).

TABLE I

Characteristics of the T Cell Clones Used in this Study

Clone designation	Antigen	H-2 restriction	Th	Source
D10	conalbumin	k	2	Janeway
AK8	conalbumin	k	2	Janeway
D8	OVA	k	2	Janeway
L16	OVA	k	2	Fitch
L17	OVA	k	2	Fitch
L18	OVA	k	2	Fitch
PL3	OVA	k	2	Fitch
CDC35	rabbit IgG	d	2	Parker
D1.1	rabbit IgG	d	1	Abbas
AR10.1	rabbit IgG	k	1	Abbas
AR10.8	rabbit IgG	k	1	Abbas
MD13.1	CRBC	d	1	Mosmann
MD13.10	CRBC	d	1	Mosmann
MD13.2.1	CRBC	d	1	Mosmann
MD264.15	CRBC	d	1	Mosmann
L2		$Mls^{a,d}$	1	Fitch
HDK1	KLH	d	1	Mosmann
GL15	OVA	k	1	Fitch
GL17	OVA	k	1	Fitch
GL18	OVA	k	1	Fitch
M6.6	OVA	k	*	Fitch
AD9	OVA	k	*	Fitch

* Produce IFN-γ and IL-4.

Results and Discussion

Preliminary experiments with $CD4^+$ T cells suggested a preferential expression of FcR on cells of the Th2 subset. To characterize these observations further, coded samples of an extensive panel of cloned T cells were analyzed for FcR expression. FcRs were found on all of the Th2 clones (Fig. 1), while FcR expression by Th1 clones was minimal or not detected (Fig. 2). Of the eight Th2 clones studied, eight bound IgA and IgM, four bound IgD, one bound IgG, and one bound IgE. Greater amounts of IgM and IgA were bound to Th2 cells compared with IgG, IgE, and IgD. Although IgM and IgA FcR expression was concordant on these T cell clones, several observations argue against a single receptor for IgA and IgM on murine T cells. (*a*) The IgM FcR on murine T cells recognizes an epitope in Cμ3, and that recognition is not inhibited by murine IgA (7). (*b*) The IgM FcR on $CD8^+$ T cells does not bind IgA (8). (*c*) The IgA FcR on $CD8^+$ T cells does not bind IgM (9). (*d*) Some murine thymic lymphoma clones are discordant for the expression of IgA and IgM FcR (M. Sandor and R. Lynch, unpublished data). (*e*) An IgM/IgA heteropentamer secreted by a murine IgM/IgA hybrid-hybridoma does not bind to T cells that do bind IgM and IgA (M. Rosenberg and R. Lynch, unpublished data).

Several laboratories have reported other surface markers that appear to be differentially expressed on Th1 and Th2 cells or their precursors, such as IL-1-Rs (10), CD45 (11), and the antigens recognized by mAbs 6C10 and 3G11 (12). Our results suggest that $CD4^+$ T cells can also be distinguished based on expression of FcRs. In three independent and blind experiments, there was a 100% agreement between the Th1/Th2 assignment made in one laboratory, on the basis of FcR expression, and the Th1/Th2 assignment made in the other laboratory. While Th1 and Th2 cells

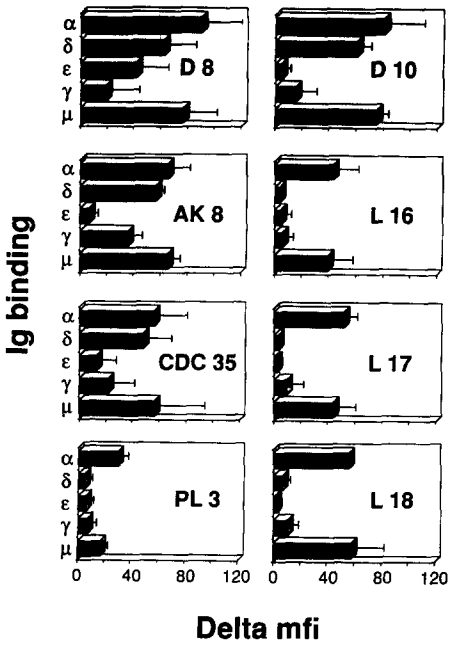

FIGURE 1. Ig binding by Th2 T cell clones (Δ mean fluorescence intensity [mfi] ± SEM).

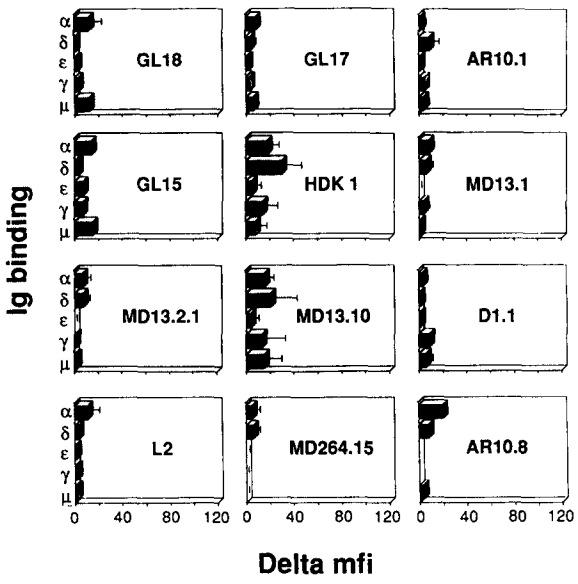

FIGURE 2. Ig binding by Th1 T cell clones (Δ mfi ± SEM).

were readily distinguished by the higher level of FcR on Th2 cells, it was clear from our data that low levels of some FcRs were present on some Th1 cells. The functional significance of high-level expression of FcR on Th2 cells remains to be determined; the observation is interesting in view of the prominent B cell helper function characteristic of Th2 cells. It is possible that expression of FcR could provide a selective mechanism for Ig-mediated signaling to antigen-activated Th2 cells. In addition, by binding to surface membrane Ig molecules on B cells, the Fc receptors on activated Th2 cells could function as accessory adhesion molecules and facilitate direct contact between Th2 cells and B cells. If the expression of FcR on Th2 cells is related to their helper function for B cells, then it might be expected that some Th1 clones would express FcR, since Th1 cells mediate some helper functions for B cells.

Recent studies have identified some $CD4^+$ clones that do not conform to the Th1/Th2 distinction that is based on the pattern of lymphokines produced. Two such clones (M6.6 and AD9) do not secrete IL-2 but do secrete IFN-γ and IL-4 (5). When

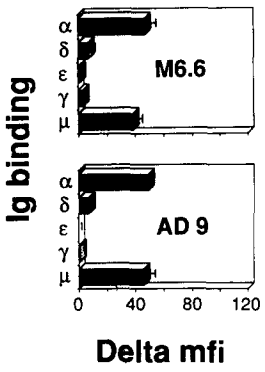

FIGURE 3. Ig binding by intermediate T cell clones (Δ mfi ± SEM).

examined for FcR expression, both of these clones exhibited high levels of IgA and IgM binding (Fig. 3) and, in this regard, are similar to Th2 clones. Preliminary results suggest that the lymphokines produced by T cell clones are not the basis for the differential expression of FcRs by Th1 and Th2 cells (Sandor, M., et al., manuscript in preparation). Th2-conditioned media does not induce FcRs on Th1 clones, and Th1-conditioned media does not inhibit FcR expression on Th2 clones. We recently found that the induction of IgA FcRs on a T cell clone was linked to cellular activation via the T3:Ti receptor (Sandor, M., et al., unpublished data). Nominal antigen presented on MHC-matched APC, or the provision of receptor-crosslinking forms of anti-T3 or anti-Ti (clonotypic) antibodies, triggered the display of FcRs. However, simply maintaining the clone in IL-2, while supporting cell cycle progression and long-term growth of the clone, did not trigger the display of FcRs. Stimulation through the T3:Ti receptor complex leads to the induction of increased intracellular Ca^{2+} concentration and the activation of the phosphoinositol cycle in Th1 cells but not in Th2 cells (13). How T cell activation through the antigen receptor leads to the induction of FcRs on Th2 cells but not on Th1 cells remains to be determined, but it could be related to differences in intracellular activation pathways that occur in Th1 and Th2 cells.

Summary

A panel of 20 murine $CD4^+$ clones was examined for the presence of surface membrane receptors for IgA, IgM, IgD, IgE, and IgG. High level expression of multiple Fc receptors (FcRs) was found on all Th2 clones. FcR expression was low or undetected on the Th1 clones. The preferential expression of FcR on activated Th2 cells suggests potential mechanisms for immunoregulatory interactions with B cells.

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