Immunology 1992 **75** 80–85

T-helper functions in lines of mice selected for high or low antibody production (Selection III): modulation by anti-CD4$^+$ monoclonal antibody

M. H. REIS, O. M. IBANEZ, W. H. CABRERA, O. G. RIBEIRO, D. MOUTON*, M. SIQUEIRA & J. COUDERC* *Laboratorio Especial de Imunogenetica, Instituto Butantan, Sao Paulo, Brazil and* **CNRS URA 1413, Laboratoire d'Immunogénétique, Section de Biologie, Institut Curie, Paris, France*

Accepted for publication 29 September 1991

SUMMARY

T-helper function was evaluated in mice genetically selected for high (H) or low (L) antibody (Ab) responsiveness to *Salmonella* flagellar antigen (Ag) (Selection III). In this Selection as opposed to what was demonstrated in Selections I, II and IVA, the interline difference was not proven to be based upon the modification of Ag processing and presentation at macrophage level. CD4$^+$/CD8$^+$ lymph node ratio is similar in HIII and LIII mice, both lines being equally susceptible to *in vivo* depletion of CD4$^+$ T cells by GK 1-5 monoclonal antibody (mAb) treatment. Nevertheless, the Ab responsiveness of the two lines was differently modulated by GK 1-5 mAb: the inhibition of Ab responses to various Ag required lower mAb doses and was long lasting in LIII as compared to the transient effect of higher mAb doses observed in HIII. LIII mice were also refractory to *Salmonella*-induced reversion of GK 1-5 mAb inhibition. Moreover, *in vitro* specific T proliferation was constantly lower in LIII, though its IL-2 production was unexpectedly similar to that of HIII T cells. Results of *in vivo* and *in vitro* experiments are thus consistent with a defective response of T-helper cells to immunogenic challenge in LIII mice.

INTRODUCTION

Mice genetically selected from outbred population for high (H) or low (L) antibody (Ab) responsiveness against complex natural Ag differ also in their responses to unrelated Ag, indicating a general modification in the mechanisms of Ab synthesis (multispecific effect).

Several selected lines of H- and L-responder mice were obtained by the use of different Ag and immunization protocols. Genetic analysis of these mice demonstrated that H or L responsiveness is under polygenic control. Moreover, comparative studies suggested the interaction of non-equivalent groups of loci in the H- and L-responder lines of each Selection.[1,2] The selective pressures exerted by the experimental selection methodology induced modifications at different levels of the pathway of Ab synthesis. It has already been demonstrated[3,4] that in Selections I, II and IV A, the main reason for low responsiveness is the higher catabolic activity of macrophages, which limits the available amounts of Ag able to trigger the immune responses in L mice. On the other hand, in H and L lines of Selections III (HIII-LIII)—subject of this article—and Selection IV, Ag-presenting cells have apparently similar catabolic activities.[5]

The selection of HIII and LIII Ab-responder lines was based on the secondary response to *Salmonella typhimurium (S.tm)* and *Salmonella oranienburg* flagellar Ag used alternately on successive generations. HIII and LIII mice were homozygous for the relevant alleles at the F_{16} generation. The genetic analysis demonstrated that the heritability was about 20%, and that genes located at 4–10 independently segregating loci were responsible for the interline difference.[6] In previous experiments, the two lines showed similar patterns of response to T-cell mitogens (Con A and PHA), allogeneic skin graft rejection and DTH reaction.[5,7]

In the present article, T-helper CD4$^+$ function was evaluated *in vivo* by inhibiting the Ab response to various Ag through pretreatment with GK 1-5 anti-CD4 mAb, the rationale being that GK 1-5 mAb dose requirement *in vivo* would assess T-cell subset potentialities in the two lines.[8,9] HIII and LIII mice were also compared for *in vitro* T-cell responses: Ag-induced proliferation and IL-2 production, as well as for their susceptibility to GK 1-5 mAb inhibition.[9,10]

Our results indicate that even if CD4/CD8 ratio is similar in both lines, T-cell proliferative response and helper effect are higher in HIII than in LIII under stimulation by various Ag.

Abbrevations: BGG, bovine gamma-globulin; CFA, complete Freund's adjuvant; CGG, chicken gamma-globulin; DTH, delayed-type hypersensitivity; FACS, fluorescent-activating cell sorter; FITC, fluorescein isothiocyanate; HoGG, horse gamma-globulin; KLH, keyhole limpet haemocyanin; LN, lymph node; LPS, lipopolysaccharide; 2-ME, 2-mercaptoethanol; PEC, peritoneal exudate cells; SRBC, sheep red blood cells; *S.tm, Salmonella typhimurium.*

Correspondence: Dr J. Couderc, CNRS URA 1413, Service d'Immunogénétique, Institut Curie, Section de Biologie, 26 rue d'Ulm, 75231 Paris Cedex 05, France.

Table 1. FACS analysis of T and B markers

	HIII		LIII	
mAb	%	mode	%	mode
GK 1-5	22·5	120	20	103
Anti-Lyt2	7	82	9	79
Anti-mouse IgG	56	451	57	459
Anti-mouse IgD	47	421	48	344

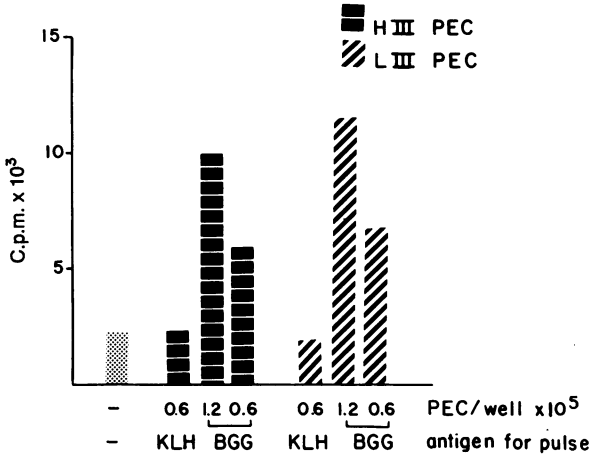

Figure 1. Stimulation of purified LN T cells (1×10^6/well) from (HIII × LIII)F_1 mice BGG-immunized 10 days before, by Ag-pulsed PEC from parental mice (IL-2 measured by [^{3}H]thymidine incorporation of CTLL in 24-hr culture supernatant).

In contrast with results in Selection I,[9] these differences are independent from the presentation efficiency of accessory cells.

MATERIALS AND METHODS

Mice

Male and female HIII and LIII mice, 2–4 months old, were bred in our animal unit at Instituto Butantan. (HIII × LIII)F1 hybrids were produced by reciprocal crosses.

Ag and immunization

For *in vivo* experiments, mice were immunized with optimal doses of SRBC (5×10^8) i.v., *S.tm* ($3{\cdot}3 \times 10^8$) inactivated with formalin i.p., or bovine gamma-globulin (BGG) (Sigma, St. Louis, MO) i.p. (100 μg), precipitated in alumen hydroxide.

Mice were bled at several time intervals as indicated.

For proliferation and IL-2 production assays, mice were injected with 100 μg of bovine (BGG), horse (HoGG) or chicken (CGG) gamma-globulin (Sigma) in 0·2 ml complete Freund's adjuvant (CFA) distributed in the four paws, and subcutaneously in the dorsal region.

Ab titration

Anti-SRBC and anti-protein Ab were titrated by direct or passive haemagglutination respectively. Proteins were conjugated to SRBC with glutaraldehyde.[11] Anti-*S.tm* Ab were titrated by agglutination. 2-ME-resistant Ab titres were determined by incubating sera in an equal volume of 0·2 M 2-ME at room temperature for 60 min, just before agglutinin titration. These Ab are referred to as IgG.

Table 2. *In vitro* Ag-dependent T-cell proliferation assay

		c.p.m./culture*		Proliferation index	
Ag†	No. of mice	HIII	LIII	HIII	LIII
HoGG	8	7284 ± 4804 (237 ± 95)	1504 ± 510 (668 ± 370)	30·7	2·3
BGG	4	30,804 ± 10,500 (2290 ± 1510)	9791 ± 7521 (1881 ± 520)	13·5	5·2
CGG	4	21,164 ± 9959 (4106 ± 2970)	8781 ± 5389 (2804 ± 1360)	5·4	3·1

* $\bar{x} \pm$SD of individual T proliferation. Numbers in brackets are $\bar{x} \pm$SD of background values.

† Ag at optimal concentration: HoGG 200 μg/ml, BGG 100 μg/ml and CGG 200 μg/ml.

T-cell proliferation assay

Mice were killed by cervical dislocation 7–10 days after Ag priming. LN were harvested, dissociated in single-cell suspension, and cultured (5×10^5 cells/well) in flat bottom microtitre plates (Falcon, Cockeysville, MD) in 200 μl Click modified medium[12] supplemented with 2% normal mouse serum (HIII + LIII v/v) and Ag for 2–5 days at 37° in humidified 5% CO_2 atmosphere.

Cultures were pulsed with 0·25 μCi [^{3}H]thymidine (specific activity 2 Ci/mmol.), and harvested 24 hr later with a Skatron automated cell harvester (Flow Labs, Irvine, Ayrshire, U.K.). Filter discs were counted in a 1221 minibeta LKB counter. Results are expressed as arithmetic means of c.p.m. from triplicate cultures.

IL-2 bio assay

1×10^6 LN cells from primed animals were cultured in microtitre plates with or without Ag in 200 μl Click modified medium. One hundred microlitre supernatant was harvested and added to 1×10^4 IL-2-dependent CTLL, suspended in 100 μl RPMI-1640 supplemented with antibiotics, 2-ME 5×10^{-5} M and 10% foetal calf serum (Gibco, Paisley, Renfrewshire, U.K.). After an 18-hr culture, 0·25 μCi of [^{3}H]thymidine were added, and CTLL harvested 6 hr later as already described.

Ag presentation assay

Resident macrophages were harvested from mice peritoneum (PEC) by extensive washing of the cavity. 1×10^6 cells were incubated for 2 hr with 200 μg/ml BGG in 2 ml Click medium, with constant shaking.

Pulsed macrophages were washed three times, 3000 rad-X-irradiated, and resuspended in Click medium. T lymphocytes from (HIII × LIII)F_1 primed animals were purified in Sephadex G10 column (Pharmacia, Uppsala, Sweden)[13] and nylon wool column successively.[14] B-cell depletion was assessed by staining with goat anti-mouse Ig-FITC serum (Institut Pasteur, Paris,

Table 3. Kinetics of T-cell specific *in vitro* proliferation

HoGG dose (μg/ml)	Line	Proliferation index* days after culture onset 2	3	4	5
20	HIII	3·73 ± 0·57	6·56 ± 1·35	5·91 ± 1·23	6·40 ± 1·52
	LIII	1·59 ± 0·33	1·84 ± 0·40	2·12 ± 0·65	2·11 ± 0·83
	H-L(*P*)†	(0·001)	(< 0·001)	(0·01–0·001)	(0·01–0·001)
200	HIII	4·18 ± 1·28	6·91 ± 1·30	9·89 ± 1·26	8·14 ± 1·81
	LIII	2·49 ± 0·95	3·32 ± 1·36	4·47 ± 1·52	3·95 ± 1·42
	H-L(*P*)†	(0·1–0·05)	(0·01–0·001)	(0·01–0·001)	(0·02–0·01)

Mice immunized with 100 μg HoGG in CFA, 8 days before cultures ($0{\cdot}5 \times 10^6$ LN cells/well).

* Mean values of four mice/group, cultures set up in triplicate.

† Calculated from Student's *t*-test.

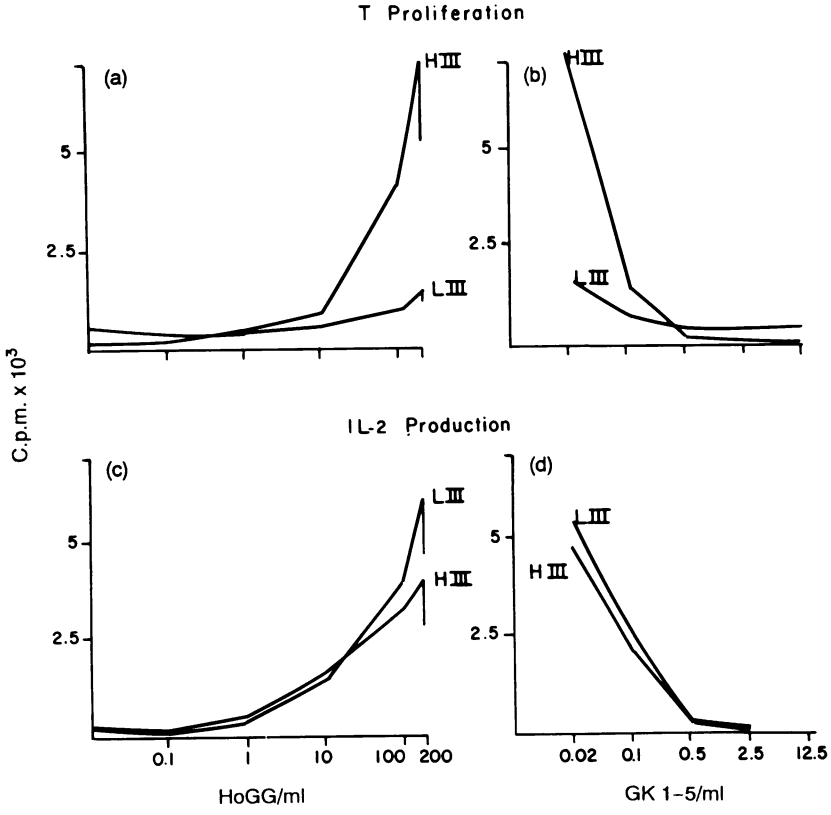

Figure 2. *In vitro* specific T-cell responses to HoGG: HIII and LIII mice immunized i.d. with 100 μg Ag + CFA 10 days before cultures [$0{\cdot}5 \times 10^6$ LN cells/well for (a) and (b); 1×10^6/well for (c) and (d)]. (a) Dose-response specific T-cell proliferation. (b) Dose-dependent GK 1-5 mAb inhibition at optimal Ag concentration (200 μg/ml), GK 1-5 mAb added at culture onset. (c) Dose-response, IL-2 production. (d) Dose-dependent GK 1-5 mAb inhibition at optimal Ag concentration (200 μg/ml), GK 1-5 mAb added at culture onset.

France). Increasing numbers of pulsed PEC were added to 1×10^6 T cells. Supernatant was harvested after 24-hr culture, and checked for IL-2 production.

Antibodies

Rat GK 1-5 mAb kindly provided by Dr Fitch (Chicago, IL) was partly purified from ascitic fluid by ammonium sulphate precipitation followed by an extensive dialysis. The protein content was measured at 280 nm.

Biotinated anti-Lyt2 and Avidine-FITC were purchased from Becton Dickinson (Mountain View, CA). Goat anti-mouse IgD-FITC was obtained from Nordic Immunology (Tilburg, The Netherlands). Mouse $(Fab')_2$ anti-Rat Ig FITC and rabbit $(Fab')_2$ anti-mouse IgG were obtained from Immu-

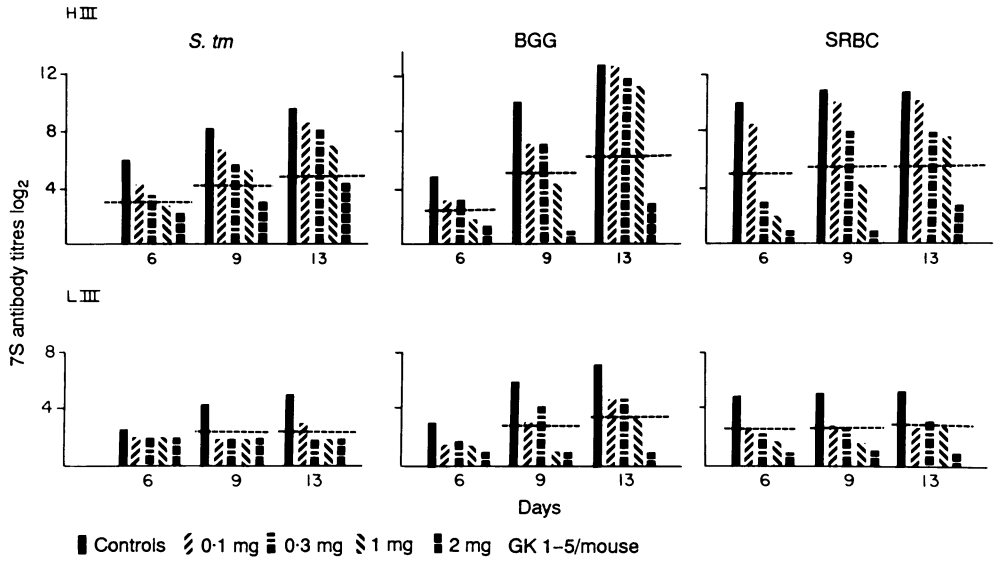

Figure 3. Inhibition of Ab production by increasing dose of GK 1-5 mAb injected simultaneously with *S.tm*, BGG or SRBC in HIII and LIII mice. IgG Ab titres on Days 6, 9 and 13. Dotted lines indicate 50% of responses in untreated immunized mice.

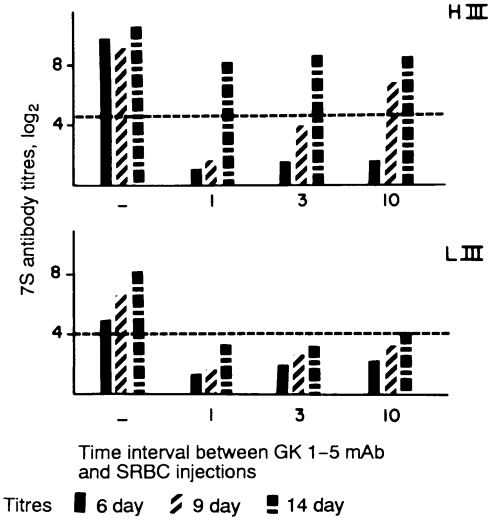

Figure 4. Duration of the inhibitory effect of GK 1-5 mAb on primary response to SRBC in HIII and LIII mice. 1 mg/mouse GK 1-5 mAb injected on Days 1, 3 or 10 before SRBC immunization. IgG Ab titres on Days 6, 9 and 14. Dotted lines indicate 50% of responses in untreated immunized mice.

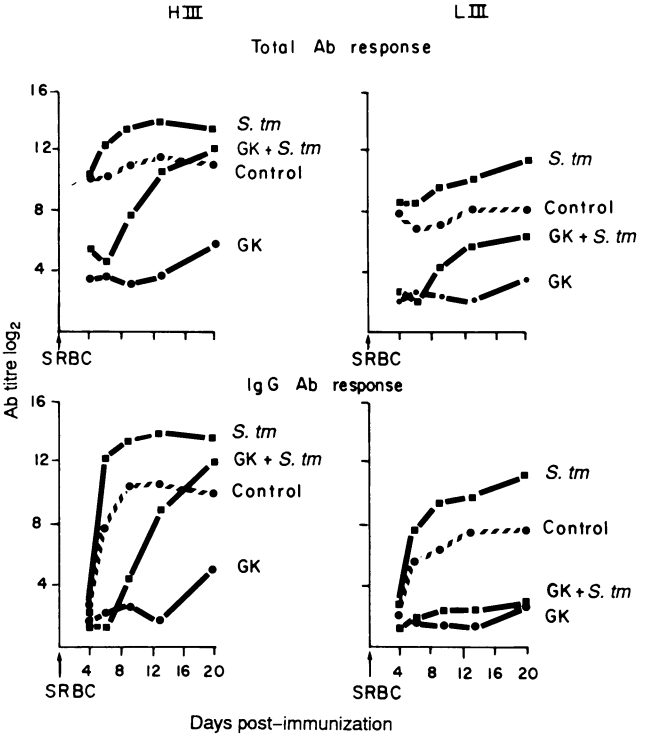

Figure 5. Kinetics of Ab response to SRBC: modulation of GK 1-5 mAb inhibition by injecting *S.tm* simultaneously with SRBC. GK 1-5 mAb 2 mg/mouse day – 1. SRBC 5×10^8/mouse i.v. *S.tm* $3{\cdot}3 \times 10^8$/mouse i.p.

notech (Marseille, France) and Cappel (Malvern, PA) respectively.

FACS analysis

LN cells were separately analysed by either direct or indirect staining in a FACScan Flow cytometer B-D using one Channel Immunofluorescence, and data collected from 10,000 cells/sample. Results are expressed as percentages of stained cells and fluorescent mode.

RESULTS

FACS analysis of lymph node cells in HIII and LIII mice

T-lymphocyte markers (CD4, CD8) and B-lymphocyte markers (membrane Ig, IgD) were used to investigate cell frequency (%) and quantitative Ag expression mode (Table 1). Results show no difference between HIII and LIII lines in percentage or mode of cell expression of these markers.

Ag presentation capacity of PEC from HIII and LIII mice to F_1 T cells

Specific activation of purified T cells from BGG immunized

(HIII × LIII)F_1 by BGG pulsed PEC of either parents was investigated (see Materials and Methods). As shown in Fig. 1, T-cell activation measured by IL-2 production is induced as efficiently by HIII and LIII Ag presenting cells, whatever the PEC/T cells ratio (1/8 or 1/16) used in these experiments. IL-2 production does not occur when PEC are pulsed with an unrelated Ag: KLH.

Specific T-cell proliferation and IL-2 production to BGG, HoGG and CGG

In order to analyse further T-helper capacities of both lines, specific proliferation and IL-2 production were tested towards several protein Ag. Experiments were performed in individual animals to prevent allogeneic stimulation, since mice were not fully inbred yet. A remarkable interline difference in T-cell proliferation capacity was observed, mainly with HoGG and BGG after 5 days of *in vitro* culture (Table 2).

The HIII-LIII difference in T-cell proliferation was independent from culture conditions. This is shown on the data upon T-cell proliferation kinetics from the second to the fifth day of culture, under stimulation with 20 and 200 μg/ml HoGG (summarized in Table 3).

These results are confirmed by the dose–response curve of proliferation to the same Ag HoGG (Fig. 2a). Surprisingly, when IL-2 production was investigated, T cells from LIII mice showed a similar or even higher IL-2 secretion when compared to HIII T cells (Fig. 2c).

IL-2 levels were maximal in 24-hr culture supernatants, and decreased by 70–80% in both lines at 48 hr (data not shown).

In a second set of experiments, the *in vitro* inhibitory effect of GK 1-5 mAb on T-cell responses was investigated by incubating cells with a constant dose of HoGG and increasing mAb concentrations. A dose-dependent inhibition of specific proliferation as well as of IL-2 production was observed (Fig. 2b, d).

Modulation of Ab production *in vivo* by treatment with GK 1–5 mAb

The effect of simultaneous injection of increasing doses of GK 1-5 mAb was investigated on Ab response to three T-dependent Ag: *S.tm*, BGG and SRBC. IgG titres were determined at Days 6, 9 and 13 after immunization. As shown in Fig. 3, HIII mice were always less susceptible to GK1-5 mAb treatment than LIII mice. At Day 6, for the three Ag tested, a 50% inhibition of Ab response was observed following injection of 1–2 mg mAb to the HIII, and of only 0·1–0·3 mg to the LIII. In addition there was a kinetic difference, since recovery from inhibition occurred faster in HIII than in LIII. Thirteen days after immunization, the degree of inhibition induced by 1 mg GK 1-5 mAb was lower than 30% for all Ag in HIII, while in LIII it remained within a 50–100% range.

The interline difference in GK 1-5 mAb effect is statistically significant ($0{\cdot}01 > P > 0{\cdot}001$). This was calculated by comparing the range of inhibition in individual mice with respect to mean responses value in the control group. It has been verified that injection of 1 mg of an unrelated rat IgG2b mAb (namely H35 17·2 mAb) had no effect in either line.

The inhibition of Ab response by GK 1-5 mAb can still be observed when the SRBC immunization is postponed until 1, 3 or 10 days after treatment (Fig. 4). Under these conditions, the Ab titres were comparably low in all groups of both lines on Day 6 post-immunization, which demonstrated that no spontaneous recovery occurred in either line.

The Ab titres rose much faster in HIII within 6–14 days after immunization. This result points out that the recovery is entirely dependent upon the immunogenic challenge.

A concomitant injection of *S.tm* (the Ag used for selection) with SRBC led to a significant adjuvant effect on anti-SRBC total and IgG responses in both HIII and LIII mice (Fig. 5). In spite of this effect, GK 1-5 mAb treatment still inhibited Ab production at the early stage of responses in both lines. In HIII, there was an escape of the inhibitory effect of the mAb, starting on the eighth day after immunization. The finding that LIII IgG—but not total—Ab responses remain inhibited by GK 1-5 mAb even under *S.tm* adjuvant effect suggests a T-dependent isotype switch-over defect in these mice.

DISCUSSION

In this article, we analyse the potentialities of T-helper compartment in lines of mice selected for high- or low-Ab production against *Salmonella* Ag (Selection III). We took advantage of GK 1-5 mAb to assess $CD4^+$ T-helper activities in the two lines during the course of immune responses.[15,16] In normal animals (Table1), subpopulations of lymphocytes (including $CD4^+$ and $CD8^+$ T cells) are present in similar frequencies in the two lines, with an identical membrane expression of the markers tested.

Functional *in vitro* studies showed that IL-2 production by HoGG primed LN cells of both lines were identical or higher for LIII mice (Fig. 2c). Similar results were obtained with CGG and BGG (data not shown).

As already established, HIII and LIII macrophages display the same capacity of Ag uptake and catabolism *in vivo*.[5] In the present study, these early data were extended using Ag presentation assay. Figure 1 shows that the activation of (HIII × LIII)F_1 primed T cells by pulsed PEC of either parent is not significantly different. Consequently, early events of T-cell activation, as measured by IL-2 production, are due to similar Ag processing and presentation to T cells in HIII and LIII mice.

Although there was no difference in IL-2 production between the two lines, the T-stimulation index was quite different: 2·3–4·5 in LIII against 10–30·7 in HIII. A possible explanation for the low proliferative capacity in LIII, in the absence of an Ag presentation defect, would be that $CD4^+$ T cells synthetize IL-2 at the onset of activation, but present a defective IL-2 receptor membrane expression[17] or affinity,[18] thus impairing their proliferative capacity and subsequent maturation steps.

The *in vivo* experiments showed that GK 1-5 mAb modulation of Ab response differed in HIII and LIII mice.

It was verified by FACS analysis that the mAb had a similar $CD4^+$ T-cell depletion effect in both lines: in fact, this subset decreased by 70 and 83% respectively in HIII and LIII mice 1 day after injection of 1 mg GK 1-5 mAb. The effective depletion is confirmed by data from Fig. 4, showing that the inhibition of IgG Ab titres is equally found in both lines in the early phase of response, whatever the time interval between GK 1-5 mAb and Ag administration. These results emphasize that during at least 16 days post-GK 1-5 mAb treatment, no recovery of $CD4^+$ population occurred in the absence of Ag administration. In contrast, the two lines differ in dose response and kinetics of the GK 1-5 mAb-mediated inhibition of Ab response: low GK 1-5

doses can induce a severe and long-lasting reduction of Ab titres in LIII, while in HIII much higher doses have only a transient inhibitory effect.

The above results suggest that the amplification of $CD4^+$ helper cells occurs faster in HIII mice under antigenic stimulation, even though one cannot exclude that the number of $CD4^+$ specific precursors might be larger in HIII than in LIII mice.

Data on the adjuvant effect of *S.tm* upon the anti-SRBC response (Fig. 5), modulated by anti-CD4 mAb, suggest that *S.tm* acts as a polyclonal B-cell activator via its endotoxin component. Similar results have been obtained using *S.tm* LPS as a co-stimulator of anti-SRBC response (data not shown): the complete inhibition by GK 1-5 mAb, of the early IgG response under LPS stimulation in both lines is in agreement with results of other authors showing that LPS also acts on T-cell subpopulations regulating the polyclonal B-cell activation.[19,20] We may postulate that this mechanism and/or a possible *S.tm* T-cell bystander effect can be large enough in HIII to reverse the mAb inhibition of IgG anti-SRBC Ab production, while LIII IgG response remains fully inhibited (see Fig. 5). These differences between HIII and LIII mice might result directly from the selective pressure, as *S.tm* had been the selection Ag in Selection III.

In conclusion there is no basic difference either in frequency or in the early stage of activation of $CD4^+$ T-lymphocyte subpopulations between HIII and LIII mice. *In vivo* and *in vitro* experiments indicate that a different regulation of T-helper subsets under antigenic stimulation could be responsible for the interline difference in Selection III Ab production.

ACKNOWLEDGMENTS

This work was supported by a grant from FAPESP (86/22-11.0). O. M. Ibanez and M. Siqueira were supported in part by CNPq. W. Cabrera was supported by FAPESP.

REFERENCES

1. Mouton D., Siqueira M., Sant'anna O.A., Bouthillier Y., Ibanez O., Ferreira V.C.A. *et al.* (1988) Genetic regulation of multispecific Ab responsiveness: improvement of 'high' and 'low' character. *Eur. J. Immunol.* **18,** 41.
2. Biozzi G., Cabrera W.H., Mouton D. & Ibanez O.M. (1982) Restricted and general polygenic regulation of Ab responsiveness. In: *Immunogenetics and Immune Regulation* (ed. B. Benacerraf), p. 31. Masson, Milan.
3. Mouton D., Bouthillier Y., Mevel J.C. & Biozzi G. (1984) Genetic selection for Ab responsiveness in mice: further evidence for inverse modification of macrophage catabolic activity without alteration of the expression of T cell-mediated immunity. *Ann. Inst. Pasteur Immunol.* **135,** 173.
4. Ibanez O., Mouton D., Oliveira S.L., Ribeiro-Filho O.G., Piatti R.M., Sant'anna O.A., Massa S., Biozzi G. & Siqueira M. (1988) Polygenic control of quantitative Ab responsiveness: restrictions of the multispecific effect related to the selection Ag. *Immunogenetics,* **28,** 6.
5. Ferreira V.C.A., Gennari M., Siqueira M., Stiffel C., Mouton D. & Biozzi G. (1985) Potentialities of immunocompetent cells in high and low Ab producing lines of mice obtained by selective breedings for responsiveness to flagellar or somatic Ag of Salmonellae. *J. Immunogenetics,* **12,** 309.
6. Sant'anna O.A., Ferreira V.C.A., Reis M.H., Gennari M., Ibanez O.M., Esteves M.B., Mouton, D. & Biozzi, G. (1982) Genetic parameters of the polygenic regulation of Ab responsiveness to flagellar and somatic Ag of Salmonellae. *J. Immunogenetics,* **9,** 191.
7. de Oliveira S.L., Ibanez O.M., Mouton D. & Biozzi G. (1985) Independent polygenic regulation of quantitative Ab responsiveness and expression of delayed-type hypersensitivity (DTH). *Clin. exp. Immunogenet.* **2,** 223.
8. Goronzy J., Weyand C.M. & Garrison-Fathman C. (1986) Long-term humoral unresponsiveness *in vivo,* induced by treatment with monoclonal Ab against L3T4. *J. exp. Med.* **164,** 911.
9. Couderc J., Bouthillier Y., Mevel J.C. & Mouton D. (1989) Evaluation of T helper function in lines of mice selected for high or low Ab production: quantitative inhibition of immune responses by anti-$L3T4^+$ monoclonal Ab. *Immunol. Letters,* **23,** 21.
10. Watts T.H., Brian A.A., Kappler J.W., Marrack P. & McConnell A.M. (1984) Ag presentation by supported planar membranes containing affinity purified I-Ad. *Proc. natl. Acad. Sci. U.S.A.* **81,** 7574.
11. Avrameas S., Taudou B. & Chuillon S. (1969) Glutaraldehyde cyanuric chlorid and tetraazotized *o*-dinisidine as coupling reagents in the passive hemagglutination test. *Immunochemistry,* **6,** 67.
12. Leonetti M., Pillet L., Maillere B., Lamthanh H., Frachon P., Couderc J. & Menez D. (1990) Immunization with a peptide having both T cell and conformationally-restricted B cell epitopes elicits neutralizing antisera against a snake neurotoxin. *J. Immunol.* **145,** 4214.
13. Lee K.C., Shiozawa C., Shaw A & Diener F. (1976) Requirement for accessory cells in the Ab response to T cell-independent Ag *in vitro. Eur. J. Immunol.* **6,** 63.
14. Julius M.H., Simpson E. & Herzenberg L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.
15. Cobbold S.P., Jayasuriya A., Nash A., Prospero T.D. & Waldmann H. (1984) Therapy with monoclonal antibodies by elimination of T cell subsets *in vivo. Nature,* **312,** 548.
16. Wofsy D., Mayes D.C., Woodcock J. & Seaman W.E. (1985) Inhibition of humoral immunity *in vivo* by monoclonal Ab to L3T4: studies with soluble Ag in intact mice. *J. Immunol.* **135,** 1698.
17. Cantrell O.A. & Smith K.A. (1983) Transient expression of Interleukin 2 receptors. Consequences for T cell growth. *J. exp. Med.* **158,** 1895.
18. Saito Y., Ogura T., Kamio M., Sabe H., Uchiyama T. & Honjo T. (1990) Stepwise formation of the high affinity complex of the Interleukin 2 receptor. *Int. Immunol.* **2,** 1167.
19. Bismuth G., Duphot M. & Theze J. (1985) LPS and specific T cell responses: Interleukin 1 (IL1)-independent amplification of Ag-specific T helper (TH) cell. *J. Immunol.* **134,** 1415.
20. Vogel S.N., Hilfiker M.L. & Caufield M.J. (1983). Endotoxin-induced T lymphocyte proliferation. *J. Immunol.* **130,** 1774.