1-1-1990

Genetic Control of Interleukin-2-Like Activity Is Distinct from That of Mitogen Response in Chickens

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Abstract

Two genetic model systems, consisting of a series of sublines differing in linkage between the B blood group (*Ea-B*) and a gene that encodes immune response to glutamic acid-alanine-tyrosine (*Ir-GAT*) and a series of highly inbred lines of chickens, were used to examine the relationship between genetic control of levels of interleukin-2-like (IL-2-like) activity and genetic control of mitogen response to concanavalin A (Con A). Results obtained by using the highly inbred lines suggested that levels of IL-2-like activity were associated with levels of mitogen response to Con A. Results obtained by using the *Ea-B/Ir-GAT* sublines, however, suggested that levels of IL-2-like activity were not associated with the mitogen response to Con A. Levels of IL-2-like activity were associated with *Ea-B* but not with *Ir-GAT,* whereas the mitogen response to Con A was associated with both. High levels of IL-2-like activity were demonstrated in birds that had low levels of mitogen response to Con A. Previous genetic events that have occurred within these sublines may have resulted in the dissociation of genetic control of levels of IL-2-like activity and the response to the blastogenesis-inducing mitogen. This demonstrates the independence of genetic control of IL-2-like activity from that of proliferative response to the inducing mitogen.

Keywords

Interleukin-2-like activity, concanavalin A, Ea-B, Ir-GAT, genetic control

Disciplines

Agriculture | Genetics | Poultry or Avian Science

Comments

This article is published as Knudtston, K. L., M. G. Kaiser, and S. J. Lamont. "Genetic control of interleukin-2-like activity is distinct from that of mitogen response in chickens." *Poultry Science* 69, no. 1 (1990): 65-71. DOI: 10.3382/ps.0690065. Posted with permission.

IMMUNOLOGY

Genetic Control of Interleukin-2-Like Activity Is Distinct from That of Mitogen Response in Chickens[1]

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(Received for publication January 4, 1989)

ABSTRACT Two genetic model systems, consisting of a series of sublines differing in linkage between the B blood group (*Ea-B*) and a gene that encodes immune response to glutamic acid-alanine-tyrosine (*Ir-GAT*) and a series of highly inbred lines of chickens, were used to examine the relationship between genetic control of levels of interleukin-2-like (IL-2-like) activity and genetic control of mitogen response to concanavalin A (Con A). Results obtained by using the highly inbred lines suggested that levels of IL-2-like activity were associated with levels of mitogen response to Con A. Results obtained by using the *Ea-B/Ir-GAT* sublines, however, suggested that levels of IL-2-like activity were not associated with the mitogen response to Con A. Levels of IL-2-like activity were associated with *Ea-B* but not with *Ir-GAT*, whereas the mitogen response to Con A was associated with both. High levels of IL-2-like activity were demonstrated in birds that had low levels of mitogen response to Con A. Previous genetic events that have occurred within these sublines may have resulted in the dissociation of genetic control of levels of IL-2-like activity and the response to the blastogenesis-inducing mitogen. This demonstrates the independence of genetic control of IL-2-like activity from that of proliferative response to the inducing mitogen.
(*Key words*: Interleukin-2-like activity, concanavalin A, *Ea-B*, *Ir-GAT*, genetic control)

1990 Poultry Science 69:65–71

INTRODUCTION

Interleukin-2 (IL-2) affects many facets of the immune response, including augmentation of the proliferation and activity of cytotoxic T (thymus-derived) cells, enhancement of gamma interferon production, enhancement of natural killer cell activity, and participation in T-dependent B (bursal-derived) cell responses (Farrar *et al.*, 1982). Because of the importance of IL-2 in the immune response, much research has focused on determining the mechanism(s) by which IL-2 activity is regulated.

Interleukin-2 activity is regulated by the inducing antigen, by suppressor factors, and by IL-2 itself (Gillis *et al.*, 1978; Smith, 1980; Kromer *et al.*, 1985; Smith and Cantrell, 1985; Reske-Kunz *et al.*, 1986). Recently, the genetic regulation of IL-2 activity has been demonstrated in rats (Lukic *et al.*, 1987). Abnormal levels of IL-2 activity have been demonstrated in individuals exhibiting altered immune responses due to genetic defects (Wofsy *et al.*, 1981; Schauenstein *et al.*, 1985; Wright *et al.*, 1987). In addition, genetic regulation of IL-2-receptor expression has been demonstrated in murine splenic T cells (Kawamura *et al.*, 1986). Although IL-2 is not considered to be restricted by the major histocompatibility complex (MHC), the cells that produce and respond to IL-2 are MHC-restricted (Wagner and Rollinghoff, 1978; Aarden *et al.*, 1979).

The assay system used to detect levels of avian IL-2 activity measures the ability of crude supernatants from concanavalin A (Con A)-activated peripheral blood lymphocytes (PBL) to promote the proliferation of a mitogen-responding population of Con A-activated lymphoblasts (Kromer *et al.*, 1984). The supernatants could, however, contain other soluble factors that may not be distinguished from IL-2, such as interleukin-1, interleukin-4, interleukin-6, and gamma interferon, which would also regulate lymphocyte proliferation (Simon *et al.*, 1979; Larsson *et al.*, 1980; Mosmann *et al.*, 1986; Habetswallner *et al.*, 1988). Because of the lack of an avian IL-2-dependent T-cell line or any other means of distinguishing these soluble factors from IL-2 in the avian IL-2 assay system, we will be referring to the activity measured by this system as IL-2-like activity.

[1]Journal Paper Number J-13252, Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, project number 2237.

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There is genetic control of the mitogenic response to Con A in the chicken (Miggiano *et al.*, 1976; Pink and Miggiano, 1977; Morrow and Abplanalp, 1981). Because it has been shown that activation of the IL-2 gene is dependent on the ligand (Weiss *et al.*, 1987), one would expect a correlation of levels of IL-2 activity and mitogen response to Con A. If there are other levels of regulation, however, levels of IL-2 activity may not be entirely dependent on the inducing mitogen; thus, IL-2 activity and mitogen response would not correlate.

The present study examined whether the levels of IL-2-like activity exhibited by genetically distinct birds were associated with the response level to the inducing mitogen. Two genetic model systems, consisting of a series of highly inbred lines and a series of *Ea-B/Ir-GAT*-defined sublines, were tested for levels of IL-2-like activity and for mitogen response to Con A.

MATERIALS AND METHODS

Animals. One recombinant line that is partially inbred [Iowa State University (ISU)-S1 line] and 13 inbred lines of chickens (*Gallus domesticus*) were used in this study. The ISU-S1 line, which is a Leghorn breed with an inbreeding coefficient of approximately 45%, has been selected for homozygosity in erythrocyte antigen B (*Ea-B*, B^1 or B^{19}) and immune-response level to glutamic acid^{60}-alanine^{20}-tyrosine^{10} (*Ir-GAT*, high or low). The inbred lines were Leghorn, Fayoumi, and Spanish breeds with inbreeding coefficients ranging from 96 to 99%. Birds were tested at 5 to 10 mo of age. Subsequent generations of birds were used to examine levels of IL-2-like activity and mitogen response to Con A. Five males and five females from each genotype of the inbred lines were examined. Ten males and 10 females from each genotype of the ISU-S1 line were examined. The birds were randomly housed in 3-m^2 floor pens with 15 to 20 birds per pen. Equal numbers of birds from each of the genotypes were randomly sampled at each collection time.

Culture Conditions. The culture medium for the IL-2 assay was serum-free RPMI 1640 (Gibco Laboratories, Grand Island, NY), supplemented with L-glutamine (50 m*M*), penicillin (100 U/mL), and streptomycin (100 μg/mL). The culture medium for the mitogen assay was the same except that 2 m*M* of L-glutamine and 5% heat-inactivated chicken serum were added. The cells were cultured at 40 C in a humidified atmosphere containing 5% CO_2.

Production of Conditioned Media. Preparation of blood samples and Con A was done as described by Knudtson and Lamont (1989). The assay used to quantitate the levels of chicken IL-2-like activity was similar to a modification by Fredericksen and Sharma (1987) of the assay developed by Kromer *et al.* (1984). The PBL were cultured at a concentration of 3.3×10^6 cells/mL in the presence of Con A (2.0 μg/mL) and 2.0×10^6 autologous red blood cells (RBC)/mL in 24-well tissue culture cluster plates at 2-mL volumes. After a 48-h incubation, the supernatants were placed into 50-mL conical centrifuge tubes. The supernatants containing IL-2 [i.e., conditioned media (CM)] were freed of cells by centrifugation [600 × *g*, 10 min, room temperature (RT)]. The CM was stored at –20 C in 1- to 2-mL aliquots in sterile vials until assayed for IL-2-like activity.

Preparation of Responder Cells. The responder cells used throughout the assay were PBL from line 19-$B^{15.1}$ birds, which yielded large numbers of responding cells. The peripheral blood lymphocytes (3.3×10^6 cells/mL) were incubated in the presence of Con A (2.0 μg/mL) for 72 h in volumes of 50 mL in 75-cm^2 or 100 mL in 150-cm^2 upright tissue culture flasks. The cells were centrifuged (600 × *g* 8 min, RT), resuspended, and incubated in RPMI 1640 medium containing .05 *M* methyl α-D-mannopyranoside (αMM) for 15 to 20 min at 40 C to competitively remove any remaining Con A from the cell surface. After one wash in Hank's Balanced Salt Solution (HBSS), the population of Con A-activated blast cells was enriched by means of density centrifugation (600 × *g*, 10 min, RT) by using a 20-60-100% discontinuous Percoll gradient. The interface between the 20 and 60% layers contained the enriched population of lymphocytes that responded to IL-2. The blasts were washed twice in HBSS and adjusted to a concentration of 2.0×10^6 cells/mL in RPMI 1640 medium containing .05 *M* αMM.

Mitogen Response Assay. Three × 10^6 PBL/mL and Con A (2 or 20 μg/mL) were cultured in 200 μL of culture medium. After a 3-day incubation, the cells were pulse-labeled for 4 h with 2 μCi of [methyl-^{3}H]thymidine

([^{3}H]dThd). The cells were collected onto glass fiber-filters by using an automated cell harvester and placed into vials containing 10 mL of scintillation cocktail [2,5-diphenyloxazole (PPO); 1,4-bis(2-5-phenyl-oxazoyl) benzene (POPOP) in toluene]. Levels of radioactivity were measured with a Packard (Downers Grove, IL) Tri-Carb 300 beta counter. Levels of mitogen response to Con A were represented as the $\log_2$ of the cpm.

Functional Determination of Interleukin-2-Like Activity in Conditioned Media. A serial 1:2 dilution of the CM to be tested was performed in triplicate in flat-bottomed, 96-well microculture plates at a volume of 100 µL/well. One hundred microliters of the responder-cell suspension were added to the CM. The control wells consisted of 100 µL of cells, CM, or culture medium plus an additional 100 µL of culture medium (RPMI 1640 with αMM) to bring the total volume to 200 µL. A reference standard (CM from GH-$B^{15.1}$, bird # 11129) was used with each test. After an 18-h incubation at 40 C in 5% CO_2, the cells were pulsed for 5 h with 1 µCi of [^{3}H]dThd. The cells were collected, and isotope incorporation was measured as described in the previous section.

Mathematical Determination of Interleukin-2-Like Activity and Mitogen Response. The value for each dilution of the test sample was expressed as the mean cpm of the triplicates after subtraction of the background cpm. One unit of IL-2-like activity was defined as the cpm of the reference standard at 25% CM. The units of IL-2-like activity for the test samples were calculated by dividing the cpm of the test sample at 25% CM by the cpm of the reference standard at 25% CM. Mitogenic response to Con A was expressed as the $\log_2$ of the cpm.

Statistical Analysis. The statistical analyses of IL-2-like activity and the mitogen response to Con A for the inbred lines consisted of two parts. An ANOVA was used to test for significant differences among the levels of IL-2-like activity or mitogen response between MHC haplotypes, lines, and sexes for all birds used in the study. In addition, individual ANOVA of differences in IL-2-like activity levels or mitogen response between MHC haplotype or line differences were conducted on available sets of matched data. The ISU-S1 line was analyzed by ANOVA to test for differences in levels of IL-2-like activity and mitogen response to Con A between birds that differed for their MHC allele or immune response to GAT. A correlation analysis between levels of IL-2-like activity and mitogen response to Con A was conducted to test whether these activities were associated. Mean levels of each genotype of these activities were compared.

RESULTS

Interleukin-2-Like Activity and Mitogen Response of Highly Inbred Lines. Each of the 13 genotypes (line-MHC allele combinations) of the inbred lines was examined for its relative level of IL-2-like activity and mitogenic response to Con A (Figure 1). Analyses of variance (not shown) for the overall effects of line and MHC allelic differences indicated that each was significantly associated with levels of IL-2-like activity and with the mitogen response to Con A. The probability of association of line differences with levels of IL-2-like activity and mitogen response, however, was more significant ($P<.001$) than MHC allelic differences ($P<.05$). The relative levels of mitogen response to Con A between the genotypes did not vary between the inducing doses (2 or 20 µg/mL). The influence of sex was not significant for levels of IL-2-like activity, whereas sex was significantly

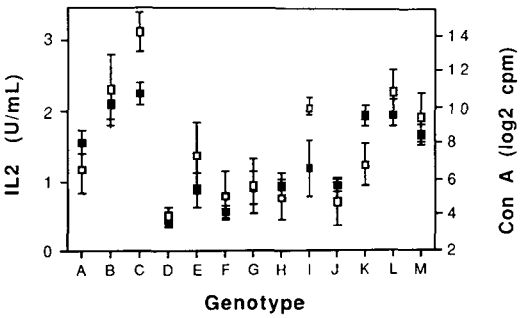

FIGURE 1. Interleukin-2-like activity (■) and mitogen response (□) to Con A (20 µg/mL) of 10 birds from each inbred genotype (mean levels and standard deviations). Genotype A = Line 8-$B^{15.1}$; Genotype B = Line 19-B^{13}; Genotype C = Line 19-$B^{15.1}$; Genotype D = Line G-B2-B^{6}; Genotype E = Line G-B1-B^{13}; Genotype F = Line GH-B^{1}; Genotype G = Line GH-B^{13}; Genotype H = Line GH-$B^{15.1}$; Genotype I = Line HN-B^{12}; Genotype J = Line HN-B^{15}; Genotype K = Line M-$B^{5.1}$; Genotype L = Line M-$B^{15.2}$; and Genotype M = Line Sp-$B^{21.1}$.

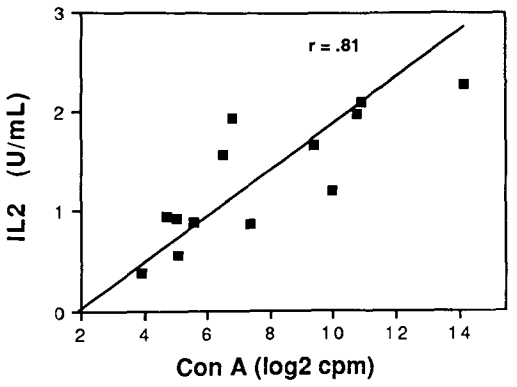

FIGURE 2. Relationship between mean levels of interleukin-2-like activity and mitogen response to concanavalin A (20 μg/mL) of 10 birds of each inbred genotype.

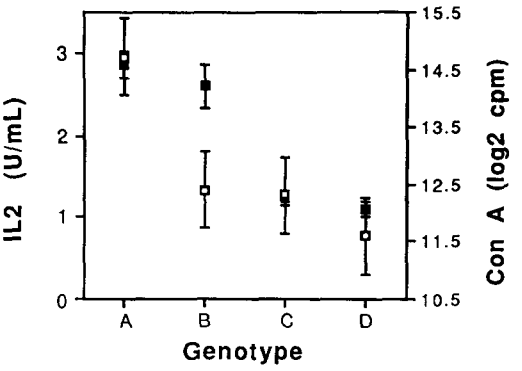

FIGURE 3. Interleukin-2-Like activity (■) and mitogen response (❐) to Con A (20 μg/mL) of 20 birds of each genotype of the ISU-S1 line (mean levels and standard deviations). Genotype A = *Ea-B*^{1}-*Ir-GAT*^{high}; Genotype B = *Ea-B*^{1}-*Ir-GAT*^{low}; Genotype C = *Ea-B*^{19}-*Ir-GAT*^{high}; and Genotype D = *Ea-B*^{19}-*Ir-GAT*^{low}

(P<.001) associated with the mitogenic response to Con A. The levels of IL-2-like activity correlate well (r = .81) with the mitogen response to Con A (Figure 2).

Interleukin-2-Like Activity and Mitogen Response of the ISU-S1 Line. The sublines (*Ea-B* by *Ir-GAT* combinations) of the ISU-S1 line were tested for their levels of IL-2-like activity and mitogen response to Con A (Figure 3). The levels of IL-2-like activity were not necessarily associated with the mitogenic response to Con A in the ISU-S1 line. Analyses of variance (not shown) indicated that levels of IL-2-like activity were significantly (P<.001) associated with *Ea-B*, but not with *Ir-GAT*. The mitogen response to Con A was not associated with either *Ea-B* or *Ir-GAT* when 2 μg/mL was used as the inducing dose. Both *Ea-B* and *Ir-GAT* were associated with the mitogen response to Con A when 20 μg/mL was used as the inducing dose. The ranking of mitogen response among the genotypes, however, was the same between the inducing doses (data not shown).

There were different numbers of nonresponders (defined as cultures generating less than 1,000 cpm) to mitogen stimulation depending upon genotype and dose of Con A. For *B*^{1}*B*^{1}-*Ir-GAT*^{high}, *B*^{1}*B*^{1}-*Ir-GAT*^{low}, *B*^{19}*B*^{19}-*Ir-GAT*^{high}, *B*^{19}*B*^{19}-*Ir-GAT*^{low} there were 6, 9, 9, and 13 nonresponders ($\bar{x}$ = 46%) to Con A at 2 μg/mL and 1, 5, 6, and 4 nonresponders ($\bar{x}$ = 20%) to Con A at 20 μg/mL, respectively. Removal of nonresponders' values did not change the genotype ranking of the mean mitogen response as compared with the analyses including both responders and nonresponders. The mean square error (MSE) within each genotype, however, was much smaller for the analysis of the mitogen response to Con A at 20 μg/mL, and this dose was used for comparisons. As with the inbred lines, sex was significantly (P<.01) associated with the mitogen response and was not associated with levels of IL-2-like activity.

DISCUSSION

Two genetic model systems (a series of highly inbred lines and the ISU-S1 line) were tested for their levels of IL-2-like activity and mitogen response to Con A. Antigen binding to its receptor is required for the activation of the IL-2 gene (Weiss *et al.*, 1987). It would therefore have been expected that levels of IL-2-like activity would be associated with the mitogen response to Con A.

Studies using the highly inbred lines support the hypothesis of an association between the mitogen response to Con A and levels of IL-2-like activity. Levels of IL-2-like activity correlated well with the mitogen response to Con A (Figure 2). These results agree with those of Pink and Vainio (1983), who suggested that high-responding cells to the mitogen would produce more IL-2 activity than

low-responding cells. In addition, it seems that more than one gene may be involved in the level of mitogen response, IL-2 activity, or both. This is shown by the different probability levels of association of IL-2-like activity and mitogen response with line (non-MHC) and MHC differences (Figure 1). This finding agrees with that of Morrow and Abplanalp (1981) who suggested that mitogen response to Con A is controlled by at least two genes, an MHC-associated gene (*ConA2*) and a non-MHC-associated gene (*ConA1*).

The results of the study of the ISU-S1 line, however, do not support the model that levels of IL-2 activity are necessarily associated with levels of response to the inducing mitogen. Rather, the results suggest the possibility that genetic control of IL-2-like activity may be distinct from that of mitogen response. High levels of IL-2-like activity were present even though mitogen response was low (Figure 3). These data, therefore, do not support the model of Pink and Vainio (1983) that low-responding cells to Con A will necessarily produce low levels of IL-2 activity.

Data from cells stimulated with high doses (20 μg/mL) of Con A were used in this experiment, because it has been shown that high doses of the mitogen were able to optimally stimulate T cells in the absence of accessory cells (Roosnek *et al.*, 1985). Because the addition of RBC to the IL-2-producing cell cultures may have enhanced antigen presentation (Kromer *et al.*, 1984), lower concentrations of Con A were able to produce optimum IL-2-like activity. Comparison was made between cells stimulated with Con A at 2 μg/mL for the IL-2-like activity assay and with Con A at 20 μg/mL for mitogenic response because these were the Con A concentrations producing optimum response in each assay. Although the ranking of Con A-blastogenic response between the genotypes using the two different doses of the mitogen did not vary, the MSE for the analysis of the 20 μg/mL study was considerably lower. The decreased MSE may be a result of the increased number of birds that responded to the higher dose of Con A.

Levels of IL-2-like activity and mitogen response can be compared among birds possessing the *Ea-B*^{1} allele in the inbred GH-B^1B^1 line and in two of the ISU-S1 sublines (Figures 1 and 3). Both the IL-2-like activity and mitogen response are low in the GH-B^1B^1 line. In the ISU-S1 line, IL-2-like activity is high in B^1B^1 birds; mitogen response is high in birds with *Ir-GAT*^{high} and low in birds with *Ir-GAT*^{low}. Previous studies have shown the GH-B^1B^1 line to have *Ir-GAT*^{low} (unpublished data). These combined data suggest that genetic control of mitogen response to Con A may be associated with the genetic control of immune response to GAT in birds with B^1B^1. This observation is not unlikely because both *Ir-GAT* and *ConA2* are associated with the MHC (Benedict *et al.*, 1975; Morrow and Abplanalp, 1981). The *Ir-GAT* was not associated, however, with the level of Con A response in $B^{19}B^{19}$ birds of the ISU-S1 line.

There are three possible explanations for the differences demonstrated in genetic associations between mitogen response and IL-2-like activity between the ISU inbred lines and the ISU-S1 line. First, because the gene pool that makes up each of the lines or sublines is very small, it is possible that, within each line, a gene controlling a particular level of IL-2 activity has segregated with a gene controlling a particular level of mitogenic response. Genes controlling IL-2 activity and the mitogenic response to Con A either may be in close proximity to each other (as suggested by the results of the study of the inbred lines) or these genes may act independently of each other (as suggested by the results of the study of the ISU-S1 line). This model seems unlikely, however, because it expects that the 13 diverse genotypes of the inbred lines all would have cosegregated the genes conferring similar relative levels of IL-2 activity and mitogen response to Con A. Also, because activation of the IL-2 gene is antigen-dependent (Weiss *et al.*, 1987), it is unlikely that the genetic associations of mitogen response to Con A and of levels of IL-2 activity within the ISU-S1 line are entirely independent.

The second explanation, which may be more probable, is that mitogenic response to Con A mediates levels of IL-2 activity, as evaluated with this functional assay system. This explanation agrees with the results of the inbred lines study, but it does not explain the results of the ISU-S1 line study unless the genetic structure of this line is understood. A previous report indicated that a genetic event (possibly a chromosomal crossover) between the *Ea-B* locus and the *Ir-GAT* locus has occurred in the ISU-S1 line, which resulted in the genotypes constituting each subline used in this study (Pevzner *et al.*, 1978). The present

data show that levels of IL-2-like activity are associated with the *Ea-B* locus but not the *Ir-GAT* locus and that the mitogen response to Con A is associated with both loci in the ISU-S1 line.

Recently, Durand *et al.* (1988) have shown that the upstream elements of the human IL-2 gene recognize at least two signals from the antigen receptor. It therefore may be possible that *ConA1* (non-MHC-associated) and *ConA2* (MHC-associated) genes each provide a signal to the IL-2 gene. Thus, a genetic event may have resulted in a different functional linkage of alleles at the *ConA2* and *Ea-B* loci, changing the level of IL-2 gene expression.

The third explanation for the differences in genetic associations between the mitogen response and IL-2 activity may be that unpurified supernatants of Con A-activated PBL were used for the source of IL-2. Perhaps other soluble factors (e.g., IL-1 and IL-4) contained in the crude supernatant may be responsible for this difference. The possibility cannot be discounted at this time.

In summary, the present study examined the association between genetic control of levels of IL-2-like activity and genetic control of mitogen response to Con A by using two different genetic model systems. The results indicated that levels of IL-2-like activity are usually associated with the level of mitogen response to Con A. In addition, however, the use of a genetic line with various linkage combinations between *Ea-B* and *Ir-GAT* has enabled the authors to determine that genetic control of IL-2-like activity as induced by Con A and genetic control of mitogenic response to Con A can be genetically dissociated.

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