# Defective T-Cell Surface Antigen Expression After Mitogen Stimulation

## *An Index of Lymphocyte Dysfunction After Controlled Murine Injury*

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**Murine spleen T-cell activation in lectin-stimulated cultures after 25% body surface area burn injury or hind-limb amputation was studied by measuring the temporal expression of cell surface markers using monoclonal antibodies and two-color flow cytometry. Lymphocyte activation has been shown to be accompanied by the appearance of new surface antigens, including Interleukin-2 (IL-2) deceptor (IL-2R) and Ia, and emergence of cells that coexpress helper ($T_h$) and suppressor ($T_s$) surface markers. IL-2R has been shown to appear early on stimulated cells, before DNA synthesis, whereas Ia appears later. Surface markers (L3T4, Lyt2, Ia, and IL-2R) were analyzed at time 0 and after 24, 48, and 72 hours of mitogen-stimulated culture. The appearance of IL-2R and Ia on $T_h$ ($L3T4^+$) and $T_s$ ($Lyt\text{-}2^+$) populations was markedly depressed after burn injury, but minimal changes were seen after musculoskeletal injury. In addition, coexpression of L3T4/Lyt2 antigens was markedly reduced in burn-derived cells. Serum from burn-injured animals caused depression of surface antigen expression by stimulated normal cells. Recombinant IL-2, when added to burn-derived cell cultures, did not increase expression of these surface markers during culture, nor did it improve proliferation.**

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FURTHER UNDERSTANDING of immunoregulatory defects that follow severe injury will require the use of increasingly sophisticated tests to measure immune functions. Better understanding of regulatory mechanisms for cell-mediated immunity (CMI) is particularly important, because CMI is often severely compromised after injury.[1,2] In addition, increasing evidence suggests that components of CMI may regulate nonspecific immunologic events, including neutrophil functions.[3-5]

Although several tests for CMI function have been used to study postinjury immune suppression, limitations in the applicability, reproducibility, and ease of use of most such tests are hindrances to their use. Two of the most commonly used assays are the measurement of incorporation of radioactive DNA precursors after mitogen stimulation or mixed lymphocyte reactions. Such tests are commonly termed "functional tests" of lymphocyte activity. However, these assays have been shown to have wide variability, and their clinical usefulness may be limited in many situations.[6-9] Furthermore, lymphocyte culture conditions may result in stimulation and proliferation of many cell populations, including "suppressor" cells; isotope incorporation by proliferating suppressor cells might thus lead to a "positive" interpretation for the test when, in fact, suppressive activity is dominating. Indeed, purified $Lyt\text{-}2^+$ cells, in the presence of accessory cells, proliferate readily upon Con-A stimulation, express IL-2 receptors, and produce IL-2.[10] In addition, DNA synthesis may not parallel other indicators of blast transformation; Rogers et al.[11] showed that many cells synthesizing DNA in culture do not undergo blast transformation, and Cotner et al.[12] demonstrated that blast transformation may result in the appearance of several surface "activation antigens" before DNA synthesis.

We therefore used a potentially more useful type of lymphocyte stimulation test to study immunity in injured mice. In addition to measuring isotope incorporation into DNA, we followed the expression of lymphocyte surface antigens during the culture period, using monoclonal antibodies and two-color flow cytometry. Stimulation and proliferation of small, resting T lymphocytes has been shown to be accompanied by the appearance of new surface antigens, which are now termed T cell activation antigens; these include the transferrin receptor,[13] the insulin receptor,[14] Ia or HLA-DR,[15] and "Tac" or IL-2 receptor.[16] In addition, cell surface coexpression of both "helper" and "suppressor" major histocompatibility complex (MHC) antigens has been shown to be an early step in T cell blastogenesis.[17] We describe in this report the lectin-

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induced expression of surface markers on murine splenic lymphocytes after controlled injury (musculoskeletal trauma or 25% body surface area burn injury). The results suggest that characterization of lymphocyte surface antigen expression after culture and stimulation may be a useful indicator of postinjury T cell dysfunction, and indicate that the block in T cell blastogenesis after severe injury occurs early in the activation process.

## Materials and Methods

### *Animals*

Female, inbred CF-1 mice, 8–12 weeks of age, from Charles River Laboratory (Wilmington, MA) were maintained in accordance with guidelines of the U.C.S.D. Animal Research Committee and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

### *Musculoskeletal Trauma*

Animals were anesthetized before trauma with brief exposure (1–2 minutes) to methoxyflurane vapor (Penthrane, Abbott Labs., Chicago, IL). The upper femur was then crushed and amputated, similar to described methods.[18,19] Animals were then injected intraperitoneally (i.p.) with one dose of morphine sulfate (m.s.) (10 mg/kg) to prolong analgesia. Control animals received both methoxyflurane and m.s. Mortality secondary to injury was negligible. In experiments not reported here, short duration methoxyflurane exposure and m.s. injection produced no detectable changes in subsequent surface antigen expression or mitogen-induced incorporation of $^3$H-thymidine by murine splenocytes.

### *Burn Injury*

Animals were anesthetized with methoxyflurane and burned by a 6-second exposure of steam to a demarcated area of the shaved dorsal skin, similar to a method previously described.[20] This produced a full-thickness burn of approximately 25% total body surface area. Animals received fluid resuscitation postburn with 3.0 cc of 0.9% NaCl i.p., as well as one i.p. injection of m.s. (10 mg/kg). Control animals were shaved and received methoxyflurane, m.s., and saline. Mortality was less than 5%, and animals maintained their weight, gradually sloughing the burn eschar over a 3–4 week period with wound healing primarily by contraction.

### *Preparation of Splenic Leukocyte Cultures*

Spleens were aseptically harvested from mice after cervical dislocation, and teased through fine stainless steel screens into 5 ml of routine culture medium (RCM) consisting of rpmi 1640-1x supplemented with l-glutamine (29.2 mg/ml − 200 mm), penicillin G (10,000 mcg/ml), streptomycin (10,000 mcg/ml), and 10% heat-inactivated fetal calf serum (FCS) (Irvine Scientific, Santa Ana, CA). Cells were pelleted by centrifugation in cold RCM. Erythrocytes were lysed by resuspending cell pellets in 2.0 ml of Tris-$NH_4Cl$ (0.017 M Tris, 0.144 M $NH_4cl$), pH 7.3 for 5 minutes on ice. Leukocytes were pelleted, washed once, counted, and resuspended in RCM at $1 \times 10^7$ cells/ml. Mitogen-stimulated cultures in a final volume of 2 ml were established in 24-well, flat-bottomed tissue culture clusters (Costar, Cambridge, MA) with $2 \times 10^6$ splenocytes, 2.5 μg or 5 μg/ml purified phytohemagglutinin or conA (5 μg/ml) and 10% FCS with medium. In selected experiments, 20 μ/ml of Il-2 (mouse, Genzyme Corporation, Boston, MA) was added at the start of culture. The cultures were incubated at 37 C in a humidified atmosphere containing 5% $CO_2$. Proliferation was assessed by setting up 0.2 ml triplicate cultures in flat-bottomed, 96-well microtiter plates. Cultures were incubated for 72 hours under similar conditions and during the final 18 hours, were pulsed with 1 μCi of $^3$H-thymidine. Cultures were harvested and processed for liquid scintillation counting.

### *Preparation of Serum*

Animals were anesthetized and immediately exsanguinated by cardiac puncture. Serum from mice 1 day, 5 days, and 10 days postburn and from normal mice was obtained and frozen at −70 C until use. The mouse serum was used in selected experiments at a concentration of 10%; FCS was not used in these experiments.

### *Lymphocyte Analysis with Monoclonal (Moab) Antibodies and Flow Cytometry*

At various times after mitogen stimulation, the splenocytes were harvested from the wells and pelleted by centrifugation before Moab labeling. Labeling with Moabs was performed as previously described.[21] The following monoclonal antibodies (Moabs) were used: anti-L3T4 and anti-Lyt-2 (rat anti-mouse, direct phycoerythrin or fluorescein conjugates, Becton Dickinson Co., Sunnyvale, CA, and in some experiments, biotinylated Lyt-2 was used), anti-Ia (rat anti-mouse, unconjugated, Hybritech, La Jolla, CA) and anti-IL-2 receptor (IL-2R) (rat anti-mouse, unconjugated, Boehringer-Mannheim, West Germany). Fluorescein-labeled goat anti-rat IgG (Becton Dickinson, Sunnyvale, CA) was used as the second antibody for the indirect assays. Incubations with the goat anti-rat IgG after anti-IL-2R binding were carried out at 37 C for 60 minutes; the remaining incubations of cells with Moabs were performed at 4 C for 30 minutes. All experiments included controls using goat anti-rat IgG-fluorescein. Wide gate settings were used, because we have previously shown that

TABLE 1. *Percent Viability of Spleen Cells in Culture*

| Culture Day | Control | Burn Injury |
|---|---|---|
| 0 | 73.4 ± 1.3 | 82.9 ± 1.2 |
| 1 | 41.4 ± 3.4 | 43.3 ± 2.8 |
| 3 | 33.6 ± 2.1 | 38.3 ± 4.1 |
| 4 | 15.7 ± 2.1 | |

burn injury results in the emergence of a larger, more granular subpopulation of splenic lymphocytes that would escape analysis if narrow gate settings were used.[21] In addition, blast transformation results in increases in cell/size granularity, reflected by increased forward/side scatter, and it has been shown that activation antigens on lectin-stimulated lymphocytes are found predominantly on larger cells.[17] Dead cells were excluded by the gate, and this was confirmed using propidium iodide staining (25 μg/ml) to identify nonviable cells.[22] The percentage of viable cells as determined by propidium iodide labeling and analysis by cytometry is shown in Table 1; the rather low percentage of viable cells after culture does not differ from that demonstrated by others, using murine cells.[23]

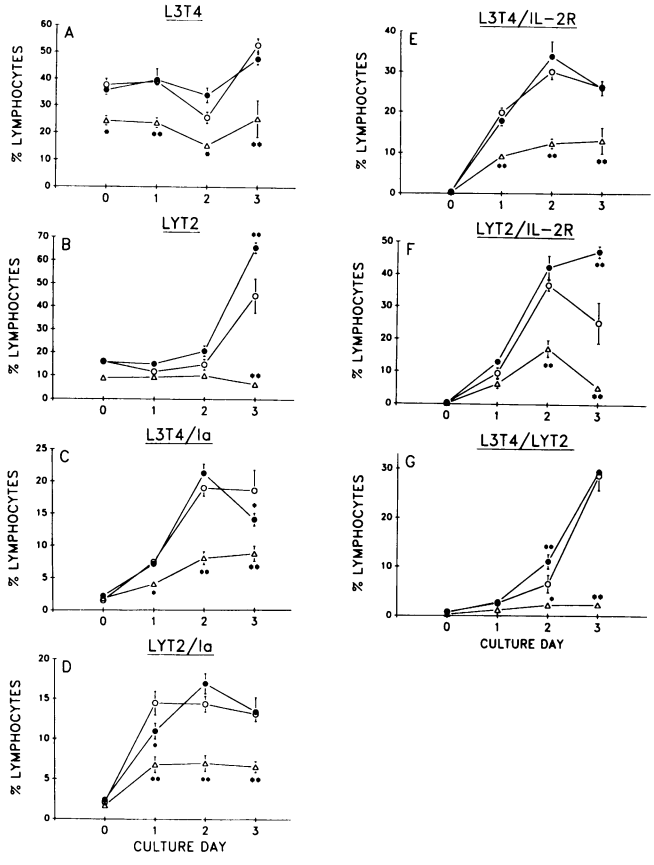


FIG. 1. Expression of splenic cell surface markers after PHA stimulation (5 μg/ml) in mice 10 days after burn injury or limb amputation (except for 1A, which shows results from Day 13 postinjury animals). Five animals were analyzed in each group. o—o, control mice; Δ—Δ, burn mice; and •—•, amputation group. Astericks indicate statistical difference between experimental and control animals (* = $p < 0.05$, ** = $p < 0.025$). All cultures were performed in presence of 10% fetal calf serum. Depression of surface marker expression in burn-injured animals was less pronounced on Day 5 postburn; however, depression was similar when Day 10, Day 13, and Day 18 postburn splenocytes were compared (all data not shown).

### *Statistical Analysis*

Cell population percentages in control and test animals were subjected to analysis of variance, and differences between groups were determined by the multiple range tests of Dunnett and Duncan. Statistical significance was assumed at $p < 0.05$. Data are expressed as mean ± SEM. Fluorescence histograms were compared by the mean fluorescence channel using FACstar (Becton Dickinson Co., Sunnyvale, CA) software to detect differences in fluorescence intensity of individual markers (proportional to surface antigen density) between control and injured mice.

## Results

### *L3T4 Surface Antigen Expression*

L3T4 surface antigen expression increased in control cells after 72 hours of PHA-stimulated culture (37.4 ± 2.3%–52.9 ± 2.5%, $p < 0.025$). Marked suppression of L3T4 was seen at the initiation and throughout the culture period, as shown in Figure 1, using cells from burned animals (10 days postburn). Results after ConA stimulation were similar (data not shown). Compared with normal animals, minimal changes in L3T4 expression were seen using lymphocytes from animals after skeletal trauma.

### *Lyt-2 Surface Antigen Expression*

Lyt-2 surface expression increased on cells from non-injured animals during PHA-stimulated culture (from 16.1 ± 1.1% to 44.8 ± 7.5% of cells, $p < 0.025$). The percentage of Lyt-2 expression was markedly depressed in cells from burn-injured animals (Fig. 1), but compared with normal animals, few changes were seen after skeletal trauma. Results after ConA stimulation were similar.

### *Ia Antigen Expression*

In cultures from control mice, Ia expression by $T_h$ and $T_s$ subpopulations markedly increased after stimulation. Burn injury resulted in marked depression in percentages of $L3T4^+/Ia^+$ and $Lyt\text{-}2^+/Ia^+3$ lymphocytes after PHA stimulation, whereas skeletal trauma produced minimal alterations (Fig. 1). Results after ConA stimulation were similar. Again, skeletal trauma produced few alterations in surface antigen expression.

### *IL-2 Receptor (IL-2R) Expression*

A small percentage (approximately 4%) of splenic lymphocytes bound Il-2 at time 0 (initiation of culture). After

culture and PHA stimulation, the overall percentage of IL-2R$^+$ cells increased to approximately 73%. As shown in Figures 1, both $T_h$ (L3T4$^+$) and $T_s$ (Lyt-2$^+$) cells from noninjured animals demonstrated increased percentage expression of Il-2R. Burn injury resulted in a marked defect in the ability of both L3T4$^+$ and Lyt-2$^+$ cells to express Il-2R, whereas musculoskeletal trauma produced minimal changes compared with normal animals. Results after ConA stimulation were similar.

### *L3T4/lyt-2 Coexpression*

Coexpression of these surface antigens has been shown to occur during mitogen-induced lymphocyte blastogenesis.[17] We noted striking increases in L3T4$^+$/Lyt-2$^+$ cell percentages in cultures from control mice. L3T4/Lyt-2 coexpression was markedly depressed after burn injury, but coexpression was minimally altered after skeletal trauma (Fig. 1).

### *Temporal Analysis of Lymphocyte Expression after Injury*

Similar changes in surface antigen expression were seen when splenocytes were studied on Days 10, 13, and 18 postburn; changes were statistically significant but less pronounced on postburn Day 5. No consistent, statistically significant changes from normal were seen on Days 5, 13, or 18 after skeletal trauma.

### *Effect of Murine Burn Serum on $^3$H-Thymidine Incorporation and Surface Antigen Expression of Normal Murine Lymphocytes*

These experiments were carried out using 10% murine serum, without FCS, to determine the effect of burn serum on lymphocyte activation and proliferation. Overall surface antigen expression, as well as $^3$H-thymidine incorporation (Table 2), in the presence of control murine serum was decreased compared with results obtained using 10% FCS, a phenomenon that has been frequently reported by others but not understood. The use of 5- and 10-day postburn murine serum resulted in marked inhibition of surface marker expression, compared with the results using normal mouse serum. Representative results are shown in Figure 2.

### *Addition of IL-2 to Burn-Derived Cell Cultures*

Recombinant IL-2 added to cell cultures at time 0 did not increase the percentage of cells from burn-injured animals expressing L3T4, Lyt-2, Ia, IL-2R or coexpressing L3T4/Lyt-2 surface antigens at 48 hours of culture, after PHA stimulation (Figs. 3A-E). In addition, addition of IL-2 did not increase $^3$H-thymidine incorporation in burn-derived cells (Fig. 3F).

TABLE 2. *Thymidine Incorporation After 72 Hours of PHA (2.5 μg/ml) Stimulation*

| | Counts per Minute |
|---|---|
| Normal animals, 10% FCS | 37400 ± 7740 |
| Burned animals, 10% FCS | 10570 ± 2881* |
| Normal animals, 10% murine serum | 2215 ± 71* |

* Different from normal animals, $p < 0.05$.

### *Fluorescence Intensity of Stimulated Cells*

Surface antigen density may reflect immunologic status. For example, other investigators have noted a wide heterogeneity in Il-2R surface density in stimulated, proliferating cell populations, and it has also been shown that the high-intensity cells account for most of the proliferative activity.[24]

After 72 hours of lectin stimulation, the fluorescence intensity, reflecting the density of surface antigens, was analyzed by histograms and expressed as the mean channel fluorescence (Table 3). The density of Ia surface antigen receptors after skeletal injury was decreased compared with control and burn cells, and increased in the burn group compared with controls. The Il-2R surface antigen receptor density on splenocytes after burn injury was decreased compared with control and trauma cells.

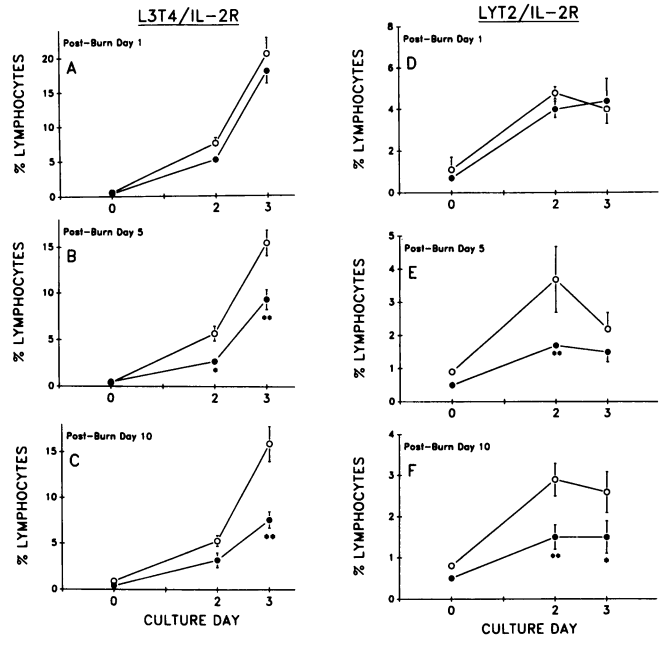


FIG. 2. Representative data showing the effects of Day 1, Day 5, and Day 10 postburn serum on PHA-induced surface antigen expression by normal murine splenocytes. Cultures were performed in presence of 10% normal mouse or postburn mouse serum, without FCS. Cell proliferation and overall surface antigen expression was decreased in the presence of mouse serum, compared with experiments using FCS. Astericks indicate statistical difference from control values (* = $p < 0.05$, ** = $p < 0.025$). o—o, normal mouse serum; •—•, burn serum.

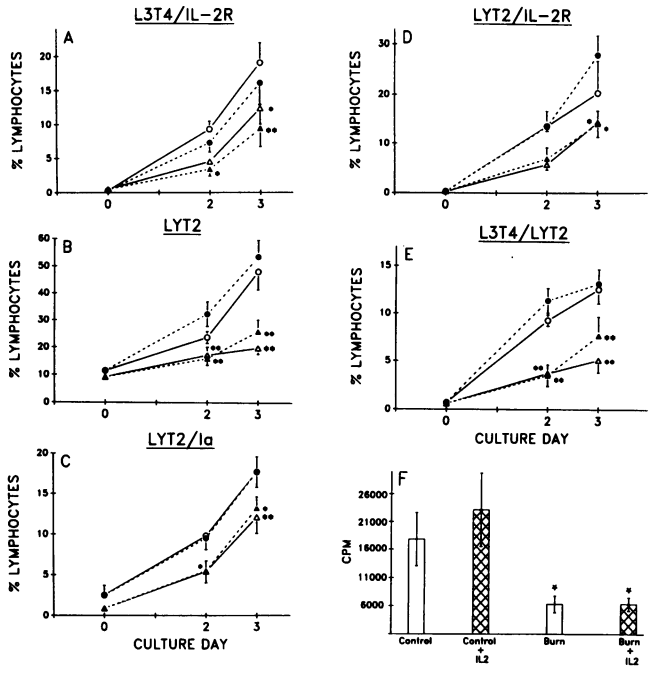


FIG. 3. The effect of exogenous IL-2 on the ability of postburn murine splenocytes to express surface antigens and incorporate $^3$H-thymidine after PHA stimulation (2.5 μg/ml). IL-2 was added to cultures at time 0 for a final concentration of 20 U/ml. In the thymidine incorporation experiments (3F), 3H-Tdr was added to 48-hour cultures; cells were harvested at 72 hours and analyzed for $^3$H-Tdr incorporation into DNA. The astericks indicate a difference compared with controls (* = $p < 0.05$, ** = $p < 0.025$). o—o, control mice; •—•, control + IL-2; Δ—Δ, burn mice; ▲—▲, burn + IL-2.

The significance of these observations remains to be determined.

### *Lympohocyte Stimulation After Moab Binding of Surface Receptors*

To determine the functional importance of the various surface antigens, spleen cell preparations from normal mice were incubated (before exposure to mitogen) with various unconjugated Moabs at Moab concentrations shown previously to be optimal for cell-labeling. Lymphocyte proliferation was measured after 72 hours of culture by addition of $^3$H-thymidine and cell harvesting. As shown in Table 4, proliferation was markedly inhibited when cells were preincubated with Moabs directed against L3T4, Lyt-2, and IL-2R antigens. No inhibition was observed when anti-Lyt-1 Moab was used, and inhibition was slight but statistically insignificant when anti-Ia was used. This study demonstrates the functional roles in lymphocyte activation and proliferation played by the surface antigens that were studied in the earlier experiments.

TABLE 3. *Mean Channel Fluorescence: Murine Spleen Cells, 5 μg/ml PHA Stimulation, 72-Hour Culture*

| | Control | Trauma | Burn |
|---|---|---|---|
| L3T4 | 453 ± 36 | 561 ± 32 | 532 ± 36 |
| LYT2 | 380 ± 45 | 477 ± 12 | 328 ± 52 |
| IL-2R | 469 ± 27 | 615 ± 27 | 418 ± 33† |
| IA | 877 ± 93 | 677 ± 20* | 1125 ± 95*† |

* Different from control, $p < 0.05$.
† Different from trauma, $p < 0.05$.

TABLE 4. *$^3$H-Thymidine Incorporation by Spleen Cell Cultures After Surface Receptor Blockade*

| Blocking Monoclonal | Counts per Minute Mean ± SEM |
|---|---|
| None (control) | 57,190 ± 7465 |
| Anti-Lyt-1 | 59,130 ± 7520 |
| Anti-Lyt-2 | 34,260 ± 5013* |
| Anti-L3T4 | 27,000 ± 4583* |
| Anti-IL-2R | 31,160 ± 4975* |
| Anti-Ia | 42,670 ± 7860 |

* Different from control, $p < 0.05$.

## Discussion

The evaluation of lymphocyte surface antigen expression has become a useful probe in immunology. Many of these molecules not only serve as phenotypic markers for functional cell classes, but some may play functional roles as well (reflected by our own data in Table 4V). For example, T4(Leu-3) and T8(Leu-2) surface proteins in the human (analagous to L3T4 and Lyt-2 in the mouse) participate in antigen recognition steps; $T4^+$ lymphocytes recognize foreign antigen predominantly in conjunction with Class II MHC molecules, whereas $T8^+$ cells recognize antigen in association with Class I MHC molecules. The T4(L3T4) molecule, in addition, plays crucial roles in both the inductive and effector phases of T cell functions; antibodies directed against T4(L3T4) antigens inhibit antigen-induced *in vitro* cell proliferation,[25] lymphokine release,[26] and some helper cell functions.[27,28] In the mouse, L3T4 and Lyt-2 molecules provide auxiliary functions during activation and/or expression of effector activity. Both antigens appear to interact with MHC molecules on accessory cells (AC) and/or target cells, and may enhance the avidity of T cell binding.[29-31] Nevertheless, the antigens appear to play different roles in some aspects. Anti-Lyt-2 antibody blocks the ConA-induced proliferation of $Lyt\text{-}2^+$ cells, whereas anti-L3T4 antibody in some experiments blocks the function of but fails to block proliferation of $L3T4^+$ cells.[10,32] Because immune dysregulation may be manifested by changes in the normal numbers or functions of T helper ($T_h$) or T suppressor ($T_s$) cells,[33,34] our laboratory has been investigating in detail these cell populations after injury.

Studies now indicate that T cell proliferation, whether initiated by antigen or lectin, is mediated by the lymphocytotrophic hormone interleukin-2, and IL-2-T cell interaction is effected via specific receptor molecules that

increase their expression after cell stimulation. T cell activation is highly complex, and a diversity of biochemical responses are correlated with different steps in the cascade of T cell activation leading to proliferation. IL-2 receptors are expressed early after lymphocyte stimulation, before DNA synthesis.[35] IL-2R expression may be transient, and receptor appearance and disappearance is mirrored by the proliferative rate of the cells.[24] We noted a marked depression in IL-2R surface expression in cultured lymphocytes from burn-injured mice; addition of Il-2 to lectin-stimulated cultures neither increased Il-2R expression nor altered expression of the other surface markers under study. Furthermore, IL-2 addition to cultures did not increase the proliferation of burn-derived lymphocytes, as measured by $^3$H-thymidine incorporation. Therefore, the failure of burn-injured cells to undergo alterations in surface antigens does not appear to be related solely to a defect in IL-2 production, although several groups have demonstrated depressed IL-2 secretion after severe injury.[36]

In studies of lectin-stimulated human lymphocytes,[12] it has been shown that the transferrin receptor and IL-2R are early antigens, appearing before DNA synthesis, and both are expressed on all proliferating cells. HLA-DR (Ia) is expressed later (after the proliferation stage begins), and does not appear on all proliferating cells. Further evidence in support of this sequence comes from experiments that show that antibodies to HLA-DR fail to inhibit mitogen-dependent T cell proliferation,[37] whereas antibodies to early activation markers reproducibly (but partially) block proliferation.[13] These results are reflected, as well, in our data of Table 4. Although we have shown that L3T4/Ia and Lyt-2/Ia antigen coexpression is markedly depressed in burn-derived cells after lectin stimulation, the studies reported here were not able to discern clear differences in the temporal expression of IL-2R and Ia antigens after stimulation. However, from the previously-cited work it appears that the block in lymphocyte activation after murine burn injury occurs early in the blastogenic response, as evidenced by the inhibition of IL-2R appearance, and before DNA synthesis and Ia expression.

The expression of $T_h$ and $T_s$ surface glycoproteins appears to be linked closely to maturation of T cells. Because both L3T4 and Lyt-2 antigens play critical roles in MHC antigen recognition, the induction or loss of either antigen may be decisive in immune regulation or differentiation. Our studies found that L3T4 expression was greatly decreased in postburn spleen cells at the time of initiation of cultures; the percentage of L3T4$^+$ cells remained constant during culture of cells from normal animals, whereas L3T4 expression remained depressed in burn-derived cells. By contrast, the percentage of cells expressing Lyt-2 greatly increased after culture of splenocytes from normal animals; this increase was almost completely blocked in burn-derived cells, whereas the percentage of Lyt-2$^+$ cells from animals after skeletal trauma actually increased during culture.

Our studies confirm those of others using human cells,[17] which demonstrate the appearance of lymphocytes that coexpress "helper" and "suppressor" antigens after lectin stimulation. The relationship of the T4$^+$(L3T4) subset to MHC recognition of Class II molecules appears more stringent than the relationship of T4 expression to helper function, because T4$^+$ lymphocytes are functionally heterogeneous and can be induced to differentiate into cytotoxic and suppressor cells.[38] Our data indicates that the overall percentage of L3T4$^+$ cells did not increase after lectin stimulation, whereas the percentage of cells expressing Lyt-2 increased markedly very early in the culture period. Therefore, it appears likely that many cells that are originally L3T4$^+$/Lyt-2$^-$ subsequently coexpress Lyt-2 antigen before cell division, as opposed to the appearance of both antigens occurring only on newly divided cells. The significance of these findings is poorly understood. However, we found that a profound decrease in the percentage of L3T4$^+$/Lyt-2$^+$ cells was a very reproducible alteration after lectin stimulation of cells from burn-injured mice. We were not able in our experiments to determine the simultaneous IL-2R or Ia expression of these cells.

Although the functional roles of these cells that coexpress "helper" and "suppressor" markers remain to be defined, they have been shown by others to highly express activation antigens such as IL-2R and have been suggested as potentially important immunoregulatory cells.[17] A majority of thymocytes have been found to coexpress both $T_h$ and $T_s$ antigens,[21,39-41] although both immunocompetent and immunoincompetent populations of $T_h^+/T_s^+$ thymocytes have been described.[42-44] A very small number of circulating lymphocytes in humans[17] and mice[21] coexpress $T_h$ and $T_s$ markers, and it has been suggested that this small subset of cells can revert to a more immature phenotype that may then function as a precursor-type cell.[17]

In conclusion, these studies demonstrate that burn-derived splenic lymphocytes are markedly deficient in their ability to express surface activation markers (IL-2R, Ia, $T_h/T_s$ antigen coexpression) after lectin stimulation, and surface antigen expression is not increased by adding IL-2 to cultures. In addition, serum from burn-injured mice, when added to lymphocyte cultures, resulted in defective lectin-induced surface antigen expression by splenic lymphocytes harvested from normal mice. Similar changes in lymphocytes from mice after musculoskeletal injury, however, were minimal. Thus, murine burn injury results in marked defects in mitogen-induced lymphocyte maturation as reflected by surface membrane antigen expression. This assay should prove useful for further study of the complex immunoregulatory events and immune dysregulation that occur after severe injury.

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