

## Effect of iron deficiency on the response of mouse lymphocytes to concanavalin A: the importance of transferrin-bound iron

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**Summary.** The *in vitro* response to Con A of lymphocytes from iron-deficient and normal mice in media containing either 10% fetal calf serum, apotransferrin or 20% iron-saturated transferrin was similar for the iron-deficient and control groups. However, the degree of proliferation in serum-free medium containing apotransferrin was significantly lower in all groups, compared to the responses in media containing either 20% iron-saturated transferrin or 10% fetal calf serum. Proliferation of lymphocytes from normal, iron-deficient or iron-repleted mice was lower in media supplemented with serum from iron-deficient mice than when serum from normal or iron-repleted mice was used. Addition of sufficient iron to bring the iron level of the deficient serum to that of normal serum significantly improved its ability to promote proliferation, while *in vivo* repletion of iron-deficient mice resulted in a restoration of normal lymphocyte responses to Con A. The proportion of cells positive for Thy 1.2, Ly 1 and Ly 2 antigens did not differ significantly between any groups of mice. Protein synthesis by cells proliferating in serum-free medium containing apotransferrin or 20% iron-saturated transferrin was the same in all groups of mice. These results indicate that decreased lymphocyte proliferative responses in iron deficiency may be due to inadequate levels of circulating transferrin-bound

iron, rather than to intrinsic defects in the cells themselves or changes in the proportions of different T-cell subsets, and that iron availability does not affect protein synthesis by proliferating lymphocytes.

### INTRODUCTION

One of the most important issues related to iron deficiency is its possible effect on cellular immune function. A number of clinical studies have investigated the effect of iron deficiency on skin reactions to antigens, or on the *in vitro* response of lymphocytes to mitogens, but these have yielded conflicting data (Jacobs, 1977). Environmental problems, such as infection, differences in sex and age between patients and controls, and deficiencies of nutrients other than iron, make it difficult to interpret human studies, and normal methods of assessing the response of lymphocytes to mitogens do not permit the control of iron levels during culture. However, it is now possible to study the *in vitro* mitogenic response of lymphocytes using serum-free conditions (Dillner-Centrelin, Hammerström & Perlmann, 1979; Brock, 1981) which allow iron levels to be accurately controlled.

This study was therefore carried out in an attempt to define more closely the effect of iron deficiency on lymphocyte proliferation, firstly by using an animal model in which the *in vitro* blastogenic response of lymph node cells isolated from mice kept on an iron-deficient diet was investigated, and secondly by

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controlling the levels of iron in the lymphocyte cultures.

## MATERIALS AND METHODS

### Animals

C3H/Bi female inbred mice aged 6–7 weeks were obtained from the departmental animal house.

### Diet

Semi-synthetic low-iron diet was prepared as follows, according to a method supplied by the Rowett Research Institute, Aberdeen, U.K. In order to make 1 kg of diet, the following quantities were thoroughly mixed: casein (200 g), commercial sucrose (548 g) and methionine (5.8 g); a further 100 g of sucrose were put aside for premixing the supplements. In addition, finely ground mineral supplement consisting of  $\text{KH}_2\text{PO}_4$  (15.7 g),  $\text{KCl}$  (1.1 g),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (5.1 g),  $\text{Na}_2\text{HPO}_4$  (6.6 g),  $\text{CaCO}_3$  (15 g) and  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$  (0.75 g) was added to the diet. The metasilicate was ground with a portion of the carrier sucrose before addition. Major trace elements ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 19.7 mg;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 176 mg and  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ , 203 mg) were powdered and mixed with another portion of the sucrose before addition of the diet. The minor trace element mixture, which consisted of finely ground  $\text{NaIO}_3 \cdot \text{H}_2\text{O}$  (1.7 mg),  $\text{NaF}$  (5.5 mg),  $\text{V}_2\text{O}_5$  (0.26 mg),  $\text{NiSO}_4$  (2.64 mg),  $\text{SnCl}_2$  (3.2 mg),  $\text{K}_2\text{Cr}_2\text{O}_7$  (14.13 mg),  $\text{Na}_2\text{SeO}_3$  (0.22 mg), mixed with sucrose (4.93 g) was prepared in bulk and stored at 4° in an air-tight bottle. Aliquots of 5 g/kg diet were mixed with more sucrose prior to addition to the diet. Water soluble vitamins (10 mg each of thiamine, pyridoxine, riboflavine and *p*-aminobenzoic acid; nicotinic acid, 30 mg; Ca-pantothenate, 20 mg, and folic acid, 5 mg) were mixed with powdered inositol (0.4 g) and sucrose (0.5% g), and added to the diet after mixing with more sucrose. Choline chloride (1 g) was mixed with vitamin  $\text{B}_{12}$  (25  $\mu\text{g}$ ) and another portion of the sucrose, and added to the diet. The water-insoluble vitamins consisting of vitamins A (8 mg), D (0.25 mg),  $\text{K}_3$  (5 mg) and E (0.3 g) were dissolved in chloroform (1 ml) and mixed with arachis oil (100 g) prior to addition to the diet. The complete diet was thoroughly mixed, stored at -4° and used within 6 weeks. As prepared, the diet contained 820  $\mu\text{g}$  Fe/kg diet and was supplemented when required with  $\text{FeSO}_4$  to give an iron content of 50 mg/kg.

### Reagents

All solutions were prepared in iron-free plastic or

acid-washed glassware using glass-distilled deionized water and, where necessary, sterilized by filtration. A 10 mg/ml stock solution of human serum albumin (Behringwerke, Hounslow, Middlesex) was prepared by dissolving albumin directly in RPMI-1640 culture medium (with 2.0 g/l  $\text{NaHCO}_3$ ; Flow Laboratories, Irvine, Scotland supplemented with 100 IU/ml penicillin and 100  $\mu\text{g}/\text{ml}$  streptomycin (Flow Laboratories) and 0.3 mg/ml L-glutamine. Human apotransferrin (Behringwerke) was dissolved in phosphate-buffered saline (PBS) containing 1%  $\text{NaHCO}_3$ . Additions of iron were made as required using iron nitrilotriacetate ( $\text{FeNTA}$ ). This was prepared by mixing ferric chloride with a four-fold molar excess of nitrilotriacetic acid. The final pH of this solution was pH 5.

Protein precipitant and ferrozine chromogen solutions used for the determination of serum and liver iron were prepared according to Brittenham (1979).

Fluorescein-conjugated monoclonal antibodies to Thy 1.2, Ly 1 and Ly 2 antigens were obtained from Becton Dickinson (Sunnyvale, CA) and used at dilutions of 1:20, 1:20 and 1:4, respectively.

### Estimation of iron

The iron content of the diet and culture medium was determined by Dr D. Halls (Biochemistry Department, Glasgow Royal Infirmary) using carbon furnace atomic absorption spectroscopy.

### Induction of iron deficiency and preparation of cell suspensions

Mice were housed individually and fed either laboratory mouse diet (ND) (Rat and Mouse No. 1 Maintenance Expanded Diet: Special Diets Services, Witham, Essex), iron-supplemented semi-synthetic diet (SD) low-iron diet (DD), or pair-fed with the iron-supplemented semi-synthetic diet (PF). All animals were given diet *ad libitum*, except the PF group which received an amount equal to the average amount consumed by the DD group on the previous day. This group was included in order to allow for possible protein-calorie malnutrition. Tap water was given to ND, SD and PF groups, while the DD group received deionized distilled water. Glass feeding dishes used for the DD group were acid-washed before use. The mice were weighed weekly and the diet was replaced daily. After 6–7 weeks, mice were killed by cervical dislocation just after the drawing of a blood sample by heart puncture. One mouse per group was killed per day over a period of 5 consecutive days. Cell suspensions were prepared by teasing the lymph nodes

under sterile conditions into RPMI-1640 medium containing 25 mM HEPES (Gibco, Paisley, Scotland) supplemented with penicillin and streptomycin. After washing once in medium (1200 r.p.m. for 45–60 seconds), the cells were resuspended in the appropriate culture medium (see below) at a concentration of  $2.3 \times 10^7$  cells/ml.

#### *Culture conditions and mitogen stimulation*

Cells were cultured in RPMI-1640 (with NaHCO<sub>3</sub>) supplemented with penicillin, streptomycin, 5  $\mu$ M 2-mercaptoethanol (2-Me) and either 10% fetal calf serum (FCS; Gibco) or 2.5% serum from C3H/Bi mice (hereafter simply referred to as mouse serum). For serum-free conditions, the medium was supplemented with 1 mg/ml human serum albumin and human transferrin (50  $\mu$ g/ml), either as apotransferrin or 20% iron-saturated. The iron content of the serum-free medium was found to be 12.5 ng/ml, which was enough to saturate 10% of the added transferrin. The cells were cultured in conical-bottomed microtitre tissue culture plates (Linbro Chemical Co., New Haven, CT), each well containing  $2 \times 10^5$  cells and concanavalin A (Con A; Miles Laboratories, Stoke Poges, Slough, Berks) at either 4, 2 or 1  $\mu$ g/ml for media containing FCS, mouse serum or transferrin + human serum albumin, respectively, in a total volume of 100  $\mu$ l. In preliminary experiments, these levels of Con A were found to give optimal proliferation in the different media. The cells were cultured at 37° in an atmosphere of 10% CO<sub>2</sub>, 90% air for 51–55 hr, or for 41–43 hr when mouse serum was used, as this was found to give optimal proliferation in this case. Proliferative responses were assayed by pulsing for 4 hr with 12.5 nCi of [<sup>14</sup>C]thymidine (Amersham International, Amersham, Bucks). The cells were harvested on a Skatron cell harvester (Flow Laboratories) and the radioactivity counted in a Packard liquid scintillation counter (Model 3320). The cultures were set up in heptuplicate, with quadruplicate Con A-negative controls.

#### *Protein synthesis*

Cells from individual mice were cultured for 55 hr in bulk at a final concentration of  $2 \times 10^6$  viable cells/ml in serum-free medium containing either apotransferrin or 20% iron-saturated transferrin and Con A (1  $\mu$ g/ml). The proliferating cells from each culture were washed twice with pre-warmed Hanks' balanced salts solution (HBSS; Gibco) at 1200 r.p.m. for 45–50 seconds, and then resuspended at a concentration of

$5 \times 10^6$  viable cells/ml in minimum essential medium (MEM) without leucine (Flow Laboratories) supplemented with penicillin, streptomycin, human serum albumin and 2-Me as described for the RPMI-1640 medium. Tritiated leucine, 10  $\mu$ Ci/ml (specific activity 160 Ci/mmol; Amersham) and 40  $\mu$ M cold leucine were added, and also apotransferrin or 20% iron-saturated transferrin (50  $\mu$ g/ml) as appropriate. Aliquots (100  $\mu$ l) were cultured in microtitre plates for 1 hr, after which 100  $\mu$ l of ice-cold PBS containing 1 mM L-leucine was added to each well and the plates chilled at 0° for 15 min. The cells were then harvested and [<sup>3</sup>H]activity was measured. The number of replicates depended on the initial density of the proliferating cells, and varied between 2 and 12.

#### *Iron repletion studies*

Three groups, each consisting of three mice, were fed either iron-supplemented semi-synthetic diet (SD: Group 1) or low-iron diet (DD: Groups 2 and 3) for 6 weeks, after which Group 3 was put on the SD diet. The mice were killed at the eleventh week. Subsequent procedures were as described above.

#### *Determination of serum and liver iron, and total iron-binding capacity.*

Serum iron was estimated by the method of Brittenham (1979), using FeNTA as standard. All assays were performed in duplicate.

For the estimation of liver iron, non-haem iron was extracted as described by Torrance & Bothwell (1980) using 0.2 g portions of liver. After digestion, 50  $\mu$ l of digest was mixed with 50  $\mu$ l deionized distilled water and, thereafter, the serum iron procedure was followed. All samples were prepared in duplicate using two different samples of liver from each mouse.

For the estimation of total iron-binding capacity, the removal of excess unbound iron was carried out by the method of Fielding (1980) and the subsequent colorimetric estimation of iron by the method of Brittenham (1979). Since insufficient serum could be obtained from individual mice for all assays, total iron-binding capacity values were obtained using pooled sera of mice of the same group, and it was occasionally not possible to perform serum iron assays on every animal in each group.

#### *Estimation of total haemoglobin*

Total haemoglobin levels were estimated in duplicate by the cyanmethaemoglobin method using a kit (Sigma Chemical Co., Poole, Dorset: Kit no. 525).

Due to the small volume of blood available, it was not possible to assay for both haemoglobin and serum iron/total iron-binding capacity on samples from the same animal.

#### Immunofluorescence

Three groups of mice were fed, either on SD (four mice), DD (four mice) or ND (three mice), for 8 weeks as previously described. Mice were then killed over a period of 1 week, and cytocentrifuge preparations of lymph node cells were made. These were fixed for 10 min in 2.5% formalin and washed in PBS for the same time before staining with the appropriate dilution of the monoclonal antibody (15  $\mu$ l per preparation) for 30–40 min in a moist box in the dark at 4°. After staining, the slides were washed three times (10 min each) in cold PBS containing 0.02% azide before mounting in PBS-glycerol (4:1). More than 300 cells from different fields taken at random were counted under phase contrast and u.v. using a Leitz Ortholux microscope, and the percentages of cells expressing each marker were calculated.

#### Statistical analysis

Analysis of variance (ANOVA) was used for lymphocyte proliferation experiments. The serum and liver iron, total iron-binding capacity, haemoglobin and surface marker figures were analysed using Scheffé's method of multiple comparisons (Scheffé, 1959).

## RESULTS

### Iron status

The haemoglobin, and serum and liver iron levels of the DD group were significantly lower, and the total

iron binding capacity considerably higher, compared with the other three groups, between which there was no significant difference (Table 1). These figures show that iron deficiency had been established in the DD group.

### Mitogenic response of lymphocytes

The degree of proliferation of lymphocytes in serum-free medium containing apotransferrin was significantly lower in all groups, compared to the responses of the cells cultured in medium containing either 20% iron-saturated transferrin or 10% FCS (Table 2). These results indicated that the iron content of the serum-free culture medium was an important factor controlling the degree of mitogenic response. In contrast, there was much less difference in the degree of proliferation when the results from different groups of mice were compared, regardless of medium. The iron content of the medium therefore did not affect the cells of the DD group very differently from those of the other groups. This indicates that the lymphocytes from the iron-deficient mice had not lost their ability to transform, due to any intrinsic defects caused as a result of iron deficiency.

In order to confirm that the degree of proliferation depended upon the amount of transferrin-bound iron in the medium, lymphocytes were cultured in medium containing either normal mouse serum, iron-deficient mouse serum or iron-deficient mouse serum to which had been added sufficient iron to bring the level up to that of normal serum. Since the previous experiment showed no difference between any control groups, the ND and PF groups were omitted. Proliferation of lymphocytes from normal or iron-deficient mice was lower in media supplemented with serum from iron-

Table 1. Iron status

Diet	Normal (ND)	Semi-synthetic + Fe (SD)	Semi-synthetic, no Fe (DD)	Pair-fed (PF)
Liver iron* ( $\mu$ g/g)	3.0 $\pm$ 0.1 (25)	2.1 $\pm$ 0.1 (24)	0.6 $\pm$ 0.02† (24)	2.2 $\pm$ 0.1 (25)
Haemoglobin (Hb)* (gm/100 ml)	13.5 $\pm$ 0.5 (5)	14.5 $\pm$ 0.6 (5)	11.4 $\pm$ 1.1‡ (5)	13.8 $\pm$ 0.9 (5)
Serum iron* ( $\mu$ g/100 ml)	230 $\pm$ 12 (14)	234 $\pm$ 16 (15)	66 $\pm$ 6† (14)	244 $\pm$ 11 (15)
TIBC§ ( $\mu$ g/100 ml)	298 $\pm$ 11 (14)	299 $\pm$ 15 (15)	429 $\pm$ 32† (14)	308 $\pm$ 4 (15)
% saturation of transferrin	77	78	15	79

\* Mean  $\pm$  SEM; number of animals given in parentheses.

† Significant difference from ND, SD and PF; no significant difference among other groups.

‡ No significant difference among any groups.

§ Three groups out of pooled sera (see text); mean of duplicate readings  $\pm$  SEM; TIBC, total iron-binding capacity.

**Table 2.** Proliferation of lymphocytes from mice on different diets, cultured with concanavalin A in media containing transferrin or fetal calf serum

Medium		<sup>[14C]</sup> thymidine incorporation (c.p.m.)			
		Normal diet (ND)	Semi-synthetic + Fe (SD)	Semi-synthetic, no Fe (DD)	Pair-fed (PF)
Serum-free + ApoTf*	+ Con A	1632 ± 425	1161 ± 550	836 ± 99	1814 ± 1020
	- Con A	135 ± 63	99 ± 42	102 ± 34	126 ± 72
Serum-free + 20% FeTf*	+ Con A	3916 ± 410	3564 ± 686	3590 ± 318	4122 ± 1210
	- Con A	186 ± 76	142 ± 95	154 ± 38	229 ± 47
10% FCS	+ Con A	3438 ± 1115	3118 ± 1020	3471 ± 757	3733 ± 921
	- Con A	710 ± 424	502 ± 436	625 ± 375	727 ± 443

Five mice per group; + Con A, seven readings; - Con A, four readings: numbers represent mean counts ± SD.

Three-way ANOVA: (i) ApoTf vs 20% FeTf: day-to-day variation,  $F = 3.37$  ( $P < 0.05$ ); diet,  $F = 4.06$  ( $P < 0.05$ ); medium,  $F = 168$  ( $P < 0.001$ ). Interactions: medium-diet, not significant. (ii) 20% FeTf vs FCS: all not significant.

\* Tf, transferrin.

deficient mice ( $3655 \pm 601$  and  $3752 \pm 601$  c.p.m., respectively) than when serum from normal mice was used ( $5569 \pm 377$  and  $5068 \pm 777$  c.p.m.). The addition of iron to the deficient serum significantly improved its ability to promote proliferation of cells from both groups of mice, the counts in cultures from normal and iron-deficient mice being  $4619 \pm 622$  and  $4969 \pm 846$  c.p.m., respectively. These results again show that lymphocytes from iron-deficient mice have a normal capacity to transform in response to mitogens and, in addition, demonstrated that serum from iron-deficient mice has inadequate iron levels to permit optimal *in vitro* proliferation.

### Protein synthesis by proliferating lymphocytes

Protein synthesis by lymphocytes which were prolifer-

ating in serum-free medium containing apotransferrin or 20% iron-saturated transferrin revealed no significant difference between any group of mice or medium (Table 3). Therefore, neither iron deficiency nor iron availability in the medium appear to affect protein synthesis by proliferating lymphocytes.

### Iron repletion

The proliferation of lymphocytes from iron-deficient or iron-repleted mice was lower in media supplemented with serum from iron-deficient mice than when serum from normal or iron-repleted mice was used (Table 4). As before, the addition of sufficient iron to bring the iron level of the deficient serum to that of normal serum significantly improved its ability to promote proliferation. Cells from iron-repleted

**Table 3.** Protein synthesis by proliferating lymphocytes

Serum-free medium containing:	<sup>[3H]</sup> leucine incorporation (c.p.m.)			
	Normal diet (ND)	Semi-synthetic + Fe (SD)	Semi-synthetic, no Fe (DD)	Pair-fed (PF)
ApoTf*	10,648 ± 3230	8699 ± 1385	8852 ± 1642	7347 ± 3326
20% FeTf*	10,156 ± 780	9183 ± 888	9604 ± 1138	8722 ± 1593

Numbers represent mean counts ± SD.

\* Three-way ANOVA: no significant difference between any groups.

**Table 4.** Effect of sera from normal, iron-deficient and iron-repleted mice on lymphocyte proliferation

Medium supplemented with serum from:	[ <sup>3</sup> H]thymidine incorporation (c.p.m.)		
	Diet: Iron-supplemented (SD)	Iron-deficient (DD)	Iron-repleted (RD)
Normal mice (SS)	1976 ± 191 (19)	ND*	2100 ± 275 (19)
Iron-deficient mice (DS)	ND	1111 ± 315 (19)	1043 ± 129 (19)
Iron-repleted mice (RS)	2200 ± 447 (19)	1882 ± 492 (19)	1909 ± 417 (19)
Iron-deficient mice + Fe (DS + Fe)	ND	ND	1946 ± 506 (18)

Number of observations are given in parentheses (six to seven per mouse); mean counts ± SD. A new batch of Con A was used, optimum concentration 1 µg/ml.

Two-way ANOVA: day-to-day variation and source effect (i.e. effect of choosing eight different combinations of cell and serum) were highly significant ( $P < 0.001$ ). The differences between all possible pairs of the eight source combinations were tested by Scheffé's method of multiple comparisons: the combination of DS-cell showed a significant difference from all other combinations. There was no significant difference among any other groups.

\* ND, not done.

**Table 5.** Percentage of T cells and T-cell subsets

Diet	Normal diet (ND)	Semi-synthetic + Fe (SD)	Semi-synthetic, no Fe (DD)
Thy 1.2 + ve	77 ± *0.5	76 ± 2.1	77 ± 1.2
Ly 1 + ve	62 ± 1.3	58 ± 3.0	64 ± 0.6
Ly 2 + ve	26 ± 0.4	25 ± 1.5	26 ± 0.9

\* Mean ± SEM.

mice proliferated as well as the cells from normal mice, and serum from iron-repleted mice was as good as normal serum and/or iron-supplemented iron-deficient serum in promoting proliferation. Thus, repletion of iron deficiency resulted in a restoration of normal lymphocyte responses to Con A, this being associated with an increase in serum iron levels.

#### T cells and T-cell subsets

The proportion of cells positive for Thy 1.2, Ly 1 and Ly 2 did not differ significantly between any groups of mice (Table 5). This suggests that iron deficiency does not affect immune responses by altering the relative proportions of different lymphocyte subpopulations.

## DISCUSSION

This study has attempted to define more closely the

effect of iron deficiency on lymphocyte proliferation *in vitro* by using an animal model in which environmental variables inherent in clinical studies are avoided, and by accurately monitoring the amount of iron in the culture medium, taking advantage of the fact that mouse lymphocytes can respond to Con A in serum-free medium (Brock, 1981). From the results, it is clear that one of the important factors in controlling the proliferation of mouse lymphocytes in response to mitogens is the content of transferrin-bound iron in the culture medium. This may explain the conflicting observations reported by earlier investigators who used sera from different sources in the culture media. This conclusion is supported by earlier studies showing that transferrin-bound iron is important for lymphocyte proliferation *in vitro* (Phillips & Azari, 1975; Brock, 1981).

Using normal and iron-deficient mouse sera, it was also found that sera from deficient mice were unable to support optimal proliferation. This agrees with the

human studies of Joynson *et al.* (1972) and Fletcher *et al.* (1975) who found a decreased *in vitro* proliferation of lymphocytes isolated from iron-deficient patients using media containing autologous sera. In the present study, supplementation of iron-deficient sera with enough iron to bring the levels back to normal increased the lymphocyte mitogenic response. It seems, therefore, that the immunological defect in iron deficiency may be due to inadequate levels of circulating transferrin-bound iron. The restoration of normal lymphocyte responses following dietary repletion observed in some clinical experimental studies (Chandra & Saraya, 1975; MacDougall *et al.*, 1975; Sawitsky, Kanter & Sawitsky, 1976; Soyano, Candellett & Layrisse, 1982) may have been dependent upon increased circulating levels of transferrin-bound (i.e. serum) iron, since serum from iron-repleted mice contained normal levels of iron and supported optimal proliferation.

It should be noted that the proportion of cells positive for Thy 1.2, Ly 1 and Ly 2 did not differ significantly between normal and iron-deficient mice, suggesting that iron deficiency does not affect immune responses by altering the relative proportions of different lymphocyte subpopulations. Kuvibidila *et al.* (1982) reported that the number of T cells in the spleens of iron-deficient mice was very much reduced in comparison to the control group, but, since the spleens were enlarged in the iron-deficient group, the total number of T cells per spleen in the iron-deficient mice may not have been very different from that of the controls.

The fact that lymphocytes from iron-deficient mice responded normally to Con A when adequate levels of iron were present in the culture media indicated that iron deficiency did not cause any intrinsic defects in the cells. This conclusion is supported by the work of Cummins *et al.* (1978) who showed that immune lymph node cells, obtained either from normal or iron- and protein-deficient donor rats, failed to induce parasite rejection in iron- and protein-deficient recipients, whereas immune lymphocytes from deficient donors could induce rejection in normal recipients. However, mitochondrial abnormalities in lymphocytes have been reported in association with iron deficiency (Jarvis and Jacobs, 1974; Jiménez *et al.*, 1982). It is possible that these morphological abnormalities may be the result of severe and prolonged iron deficiency, and do not develop in the comparatively short time for which the experimental animals were kept on an iron-deficient diet in the present study.

Although DNA synthesis by lymphocytes is dependent on transferrin-bound iron, protein synthesis by proliferating cells was not affected in the present study, either by iron deficiency or by the availability of iron in the culture medium. This suggests that there was no intrinsic defect in the ability of these cells to synthesize protein and that protein synthesis, unlike DNA synthesis, does not appear to be directly dependent upon the availability of transferrin-bound iron.

In conclusion, transferrin-bound iron appears to be a controlling factor for *in vitro* lymphocyte proliferation, but it is difficult to predict how this compares with the *in vivo* situation. Although, under normal conditions, there is always a constant supply of circulating transferrin-bound iron, in iron deficiency the saturation of transferrin with iron is very much reduced and, in a localized area such as a lymph node, lymphocyte proliferation may utilize transferrin-bound iron faster than it can be replenished, resulting in impairment of the immune response. This, together with tissue abnormalities which may result from prolonged iron deficiency, may have important implications for the pathogenesis of malignant disease and chronic infection.

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